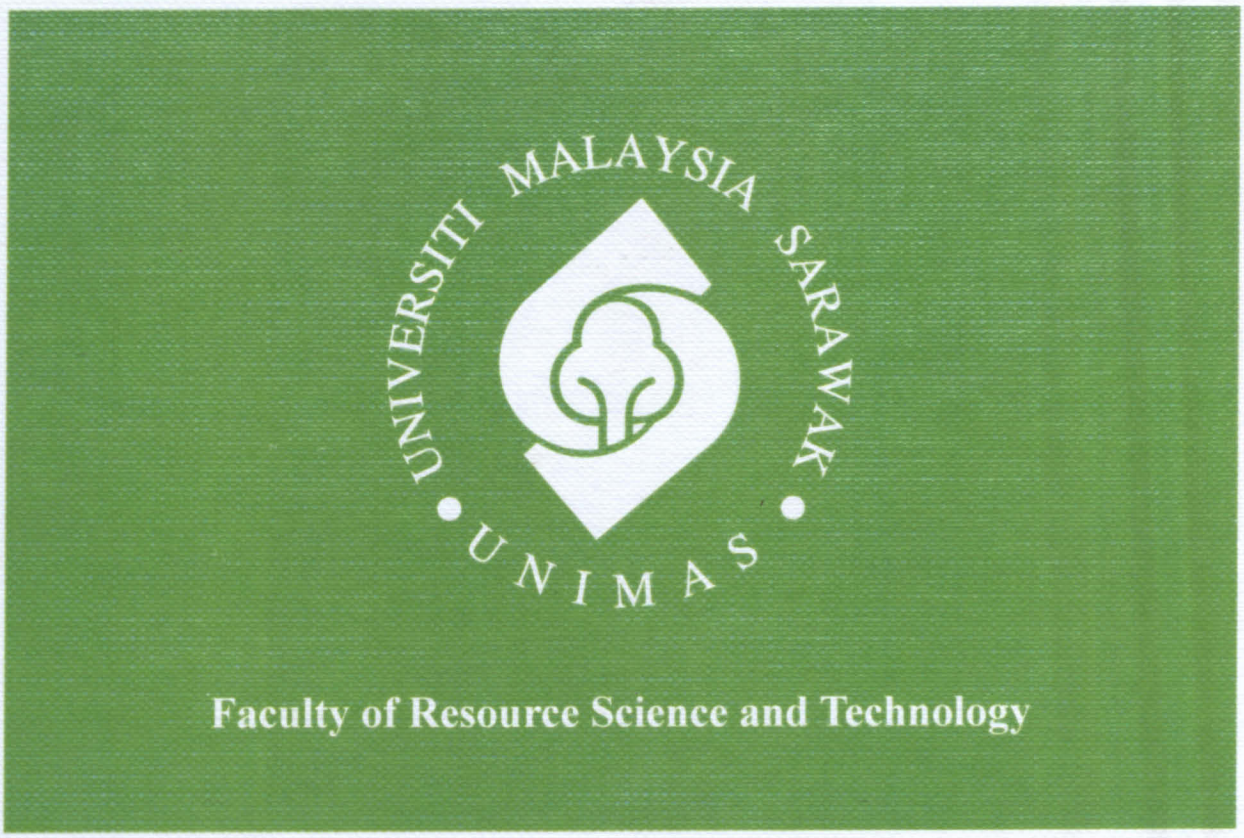

Screening of antibacterial activity of *Etlingera littoralis* stem extract

Nor Amirah binti Mohd Rosli

Bachelor of Science with Honours
(Resource Biotechnology)
2018

Borang PTA4

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Screening of antibacterial activity of *Etlingera littoralis* stem extract

Nor Amirah binti Mohd Rosli

The dissertation is submitted in partial fulfillment of requirement for degree of Bachelor Science with Honours in Resource Biotechnology

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

2018

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Final Year Project Report ☑

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This declaration is made on the 7th day of JUNE year 2018

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Screening of Antibacterial Activity of *Etlingera littoralis* stem extract

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ABSTRACT

Etlingera littoralis is a common ground species of family Zingiberaceae. It is widely distribute in Asia and Pacific region. *E.littoralis* have various traditional use even the young shoot, flower buds and the fruits are consumed by the indigenous communities. Besides, the cores of young stems of *E.littoralis* which also known as 'Tepus' are edible and very popular food among Dayak community in Sarawak. However, there are lack of specific research about *E.littoralis* stems especially on antibacterial activity. The aim of this study was to get some preliminary data by determine the antibacterial activity of *E.littoralis* stems. The fresh stems of *E.littoralis* was screened for antibacterial activity in both gram positive and gram negative bacteria. The extraction of the stems used was maceration process which involve methanol, ethanol and acetone solvent. While, the antibacterial activity was used agar well diffusion method. All extracts showed antibacterial activity except for acetone extract. The antibacterial activity of *E.littoralis* stems in different solvent showed different sensitivity against the test bacteria. However, the methanol extract showed the highest zone of inhibition of antibacterial activity (8.33-10.67 mm) but only in Gram-positive bacteria at 400 mg/ml concentration. Gram-positive bacteria were more sensitive compared to Gram-negative bacteria. Besides, ethanol extract showed the inhibition zone against all tested bacteria and the highest zone were at 400 mg/ml concentration with the diameter ranging from 8.33-10.00 mm. In this study, it showed the antibacterial potential on *E.littoralis* stems which could be use to create a new antibiotics drug.

Key words: *E. littoralis* stem, antibacterial activity, maceration process, agar well diffusion method

ABSTRAK

Etlingera littoralis adalah spesies asas keluarga Zingiberaceae. Ianya meluas di rantau Asia dan Pasifik. E.littoralis mempunyai pelbagai kegunaan tradisional dan pucuk muda, tunas bunga dan buah-buahan dimakan oleh masyarakat pribumi. Selain itu, batang E.littoralis yang juga dikenali sebagai 'Tepus' boleh dimakan dan adalah makanan yang sangat popular di kalangan masyarakat Dayak di Sarawak. Walau bagaimanapun, terdapat kekurangan penyelidikan khusus mengenai batang E.littoralis terutamanya pada aktiviti antibakteria. Tujuan kajian ini adalah untuk mendapatkan beberapa data dengan menentukan aktiviti antibakteria batang E.littoralis. Batang segar E.littoralis telah digunakan untuk mengkaji aktiviti antibakteria oleh bakteria Gram-positif dan Gram-negatif. Pengekstrakan batang adalah menggunakan proses maserasi yang melibatkan pelarut metanol, etanol dan aseton. Sementara itu, aktiviti antibakteria adalah menggunakan kaedah difusi agar. Kesemua ekstrak menunjukkan aktiviti antibakteria kecuali ekstrak aseton. Aktiviti antibakteria terhadap E.littoralis dalam pelarut yang berbeza menunjukkan sensitiviti yang berbeza terhadap bakteria ujian. Bagaimanapun, ekstrak metanol menunjukkan zon tertinggi perencatan aktiviti antibakteria (8.33-10.67 mm) tetapi dalam bakteria Gram-positif sahaja. Bakteria Gram-positif lebih sensitif berbanding bakteria Gram-negatif. Selain itu, ekstrak etanol menunjukkan zon inhibisi terhadap semua bakteria yang diuji dan zon tertinggi berada pada kepekatan 400 mg/ml dengan diameter antara 8.33-10.00 mm. Dalam kajian ini, ia telah menunjukkan potensi antibakteria pada batang E.littoralis yang boleh digunakan untuk mencipta ubat antibiotik baru.

***Kata kunci**: Batang E. littoralis, aktiviti antibakteria, proses maserasi, kaedah difusi agar*

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List of abbreviation

B. cereus	*Bacillus cereus*
CFU	Colony-forming unit
DMSO	Dimethyl sulfoxide
E. coli	*Escherichia coli*
E. elatior	*Etlingera elatior*
E. littoralis	*Etlingera littoralis*
E. maingayi	*Etlingera maingayi*
E. sayapensis	*Etlingera sayapensis*
MHA	Muller-Hinton Agar
MHB	Muller-Hinton Broth
NA	Nutrient agar
P. aeruginosa	*Pseudomonas aeruginosa*
S. aureus	*Staphylococcus aureus*
SD	Standard deviation

1.0 Introduction

The genus *Etlingera* is one of the largest in the ginger family Zingiberaceae. The scientist have discover about 1200 species which predominantly found in Asia and the Pacific regions. The highest concentration of this genus and species in Asia including Indonesia, Malaysia, Singapore, Brunei, Philiphines, Thailand and Papua New Guinea. According to Yeats (2012), the *Etlingera* specimen was originally collected by Johann Gerhard KÖnig in 1779 in southern Thailand. The Zingiberaceae are the ground plant which commonly found in the primary and secondary tropical forest. However, the larger species are tall forest plants which may reach 6 m in height (Khaw 2001; Chan *et al.*, 2007). Many of them grow in humid and under shade of taller trees but, some of the species can fully expose to the sun.

Etlingera littoralis is one of the species in genus *Etlingera* and widely distributed in Asia especially in Gulf of Thailand and Andaman Sea Coasts. This plant height is up to 3 m tall and they have a very bright red flowers at the ground level. In Borneo, they have a red flower and sometimes a yellowish in the margin while in Southern Thailand, the flower is red but the margin is yellow in color (Chongkraijak *et al.*, 2013). In Sabah, Malaysia, the young shoots, flower buds and fruits of *E. littoralis* are consumed by the indigenous communities as condiment, eaten raw or cooked (Noweg *et al.*, 2003; Chan *et al.*, 2007). Besides, the cores of young stem are edible and the local name of the stem known as 'Tepus' which is popular in Dayak community in Sarawak (Pilo, 2013).

According to Yeats (2012), the Zingiberaceae are important in pharmacological research as they validating the antioxidant and antibacterial that will be use in medicinal and cosmetics. The antibacterial are anything that can kill or destroy the specific bacteria because it have the ability to growth and replicate. The antibiotic drug is one of the

example that have the antibacterial property. Antibacterial from plant extracts now become popular among clinical microbiologists. According to Cowan (1999), the content of the plant extracts which is the phytochemical will be a good source of antibacterial drugs instead of antibacterial drugs that derived from microorganism.

Some of the species of *Etlingera* have been well studied. For instant, *Etlingera elatior* are well known in its medical properties. According to Jackie *et al.,* (2011), the fruits are use to treat ear ache, leaves use to healing the wound, inflorescence have high antioxidant and flower shoot have antibacterial, cytotoxic and anti-tumor. Many previous study on antibacterial activity were towards *E. elatior*. However, there is lack of research published on the *Etlingera littoralis* especially on the antibacterial activity of the stem part of the plant. Therefore, this study is to get new source of antibacterial agent on the *E. littoralis* stem. The objective of this study are to extract the *E.littoralis* stem by using methanol, ethanol and acetone in maceration process and to evaluate the antibacterial activity from the crude extract of *E.littoralis* stem against selected gram positive and gram negative bacteria by using agar well diffusion method. Since the previous study have shown the antibacterial activity on *E. littoralis* leaves, the use of stem in this study may enlighten the antibacterial potential of other part of *E. littoralis*.

2.0 Literature review

2.1 *Etlingera* genus

The Zingiberaceae are important which is in pharmacological research as it can use to search for antioxidant and antibacterial healing properties for medicinal (Yeats, 2012). In the ginger family Zingiberaceae, *Etlingera* is one of the largest genus. This *Etlingera* Giseke are tall forest plants which is the larger species of this genus can reached six meter in height (Khaw, 2001; Chan *et al.,* 2007). According to Sirirugsa (1999), in Thailand, the flowers of *Etlingera elatior* and *Etlingera maingayi* are eaten as vegetables. Fruits and cores of young stems of *Etlingera littoralis* are suitable for eating raw or cooked. A total of 15 *Etlingera* species have been reported in Peninsular Malaysia (Lim, 2001). *E. littoralis* is one of the species that have been found in Malaysia.

Figure 2.1: *Etlingera littoralis* stem.

Besides, there are quite number of studied that have been done on these 15 species. *E. elatior* inflorescence have high antioxidant and the flower shoot have antibacterial properties (Jackie *et al.,* 2011). According to Mahdavi *et al.,* (2017), the rhizomes oil and the leaves oil of *Etlingera sayapensis* have shown the antimicrobial activity but the stem oil did not run because it was not enough for the test as they use it to identify the antioxidant activity. He also stated that there is a possibility for the stem oil to show the antimicrobial activity since there is an outstanding similarities in the major groups of compound from different *Etlingera* species. According to Chan *et al.,* (2007), leaves of *E. littoralis* have antioxidant and antibacterial properties which have great potential to be develop into natural preservatives and herbal products. For this study, the *E. littoralis* stem will be use for the screening of antibacterial activity.

Figure 2.2: Cores of young stem of *E. littoralis* (Tepus) used for the screening for antibacterial activity.

2.2 Stem extraction

Extraction is separation of active compound which is between soluble and insoluble using selective solvent. According to Nascimento (2000), medicinal plant would be the best source to obtain drugs and about 80% of the individuals from developed countries use traditional medicine which has compounds derived from medicinal plant. Among the natural plant-derived products (such as green tea, herbal decoctions, or herbal medicines), flowers is one part of the plant that have attained high priority and found various applications (Voon *et al.*, 2012). In in previous studied, the leaves of *Etlingera pavieana* have high antibacterial activity, followed by stem and rhizome (Naksang and Rachtanapun, 2015). Therefore, stem material also can be taken as an alternative source to screen and determine the antibacterial agent. Furthermore the extraction method used are also similar to the flower and leaves extraction.

For this study, the extraction method used is maceration process. According to Azwanida (2015), maceration is a technique that have been used in wine making and also in medicinal plant research. Maceration process involve soaking plant material whereby the powdered plant materials and a solvent will be mix in the stopped container and allow to stand at room temperature with frequent agitation for at least three days. This process can soften and break the plant's cell wall to get the soluble phytochemical. Besides, maceration are the easiest, simple and less costly method instead of modern extraction method (Vongsak *et al.*, 2013).

2.3 Antibacterial activity

Antibacterial is anything that destroys bacteria or suppresses their growth or their ability to reproduce. According to Ruban and Gajalakshmi (2012), natural products are popular in modern clinical drugs because it play an important role in the drug development programs for pharmaceutical industry like herbal treatment, which can treat disease that cause by the multi drug resistant bacteria. The pure compounds of plant extracts have provide suitable additives and also drug treatments. This is because the pure compound of plant extract contain the different range of chemical diversity (Klancnik *et al.*, 2010). Plant extract with known antibacterial properties is suitable for therapeutic treatment especially to those that related to control the antibiotic resistant bacteria (Nascimento *et al.*, 2000).

Besides, antibacterial also important to control food poisoning disease and preserve foodstuff (Mostafa *et al.*, 2018). Food poisoning is an illness that caused by the consumption of food or water that are associated with bacterial contamination especially Gram-negative bacteria while Gram-positive bacteria as causal agent for food borne disease. The natural product like plant extract can be a new source to cure the food poisoning disease as many antibacterial agent may have resistant to some of the bacteria that cause food poisoning.

Furthermore, plant extract have been use in research to develop potentially effective, healthy safer and natural food preservative. Food preservative is an alternative for the food to prevent the degradation by bacteria, prevent the growth of bacteria or fungi and make it long lasting. The plant extract is natural sources of antibacterial agents for food preservation because it is easy degradable and nutritionally safe (Mostafa *et al.*, 2018).

2.4 Agar well diffusion method

According to Balouiri *et al.,* (2016), agar well diffusion method is widely used to measure the ability of the plant or microbial extracts to inhibit the bacteria growth. In this study, agar well diffusion method is use to measure the antibacterial activity against Gram-positive and Gram-negative bacteria. Agar well diffusion method allow to study a vast number of samples, microorganisms and antibacterial agent. This is because, it is a simple, rapid and inexpensive screening method (Oses *et al.,* 2016).

According to Chigurupati *et al.,* (2017), the test bacterial inoculum were uniformly spread by sterile cotton swab on Muller-Hinton Agar (MHA) plate. Then, 6 mm diameter of wells cut into the surface of agar (Jahangirian *et al.,* 2013). Agar well diffusion involves the extract of different concentration add to each of the wells that have been punched on the agar plates (Valgas *et al.,* 2007). A volume of 50 µl of every concentration of plant extracts were added to each of the four wells (Chigurupati *et al.,* 2017). According to Oses *et al.,* (2016), the diffusion of extract in agar medium will inhibit the growth of bacteria strain tested and lead to the formation of clear zones. The zones of inhibition produced by the test organisms indicated their susceptibility to the plant extracts (Cheruiyot *et al.,* 2009). This inhibition of bacteria growth was measured in millimeter.

2.5 Test bacteria

This research is to detect antibacterial activity in *Etlingera littoralis,* whereby it involve common bacteria species. Different types of bacteria which are Gram-positive bacteria and Gram-negative bacteria will be uses. Two species from Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) and two species from Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). *S. aureus* is Gram-positive and have

tough cell wall because it consist of peptidoglycan. It can cause local infection of the skin, nose, urethra, vagina and gastrointestinal tract (Harris *et al.,* 2002). *B. cereus* is Gram-positive, aerobic to facultative and spore-forming rod which will lead to respiratory tract infection and nosocomial infection instead of the common illness which involve in food poisoning (Bottone, 2010).

Besides, *E. coli* is Gram-negative, facultatively anaerobic and rod-shape bacteria which can cause urinary tract infection, colitis, diarrheal disease and some strain may cause cancer (Blount, 2015). While, *P. aeruginosa* is Gram-negative, aerobic and rod-shape bacteria. It can cause several infection like pneumonias, nosocomial, urinary tract, bloodstream and throat infection (Lister *et al.,* 2009). According to Chanda *et al.*, (2013), these microorganisms are important as they can cause several infection, food borne disease, skin infection and spoilage which it need active therapeutic agent to overcome them.

3.0 Materials and method

Etlingera littoralis stem was extracted by using maceration process and for the screening of antibacterial activity, agar well diffusion method have been used. The procedure that have been applied in this study is summarized in the (Figure 3.1).

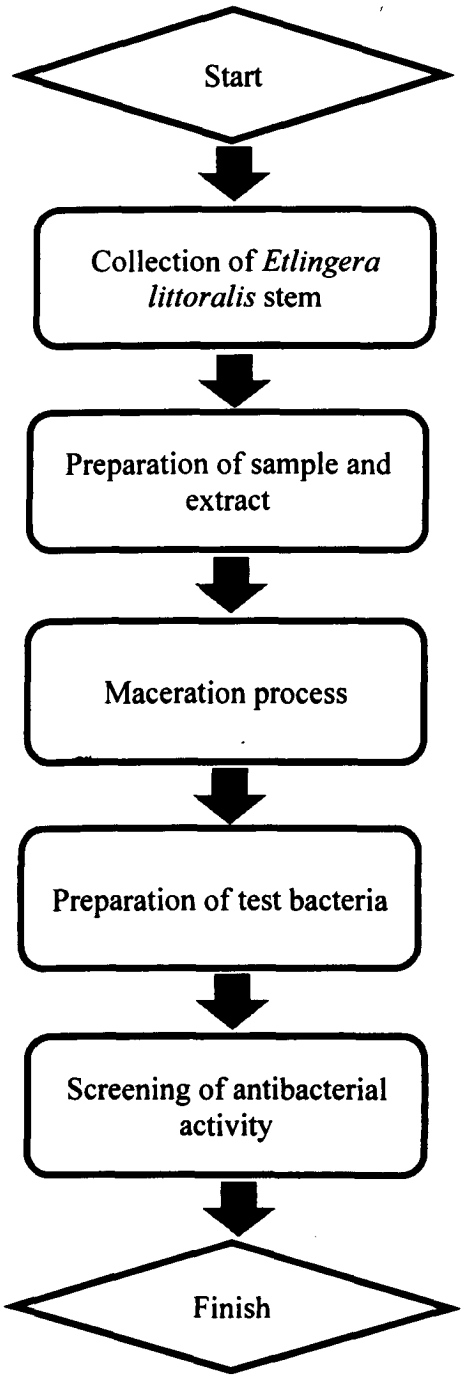

Figure 3.1: Flowchart of methodology

3.1 Collection and preparation of sample

The sufficient amount of fresh sample of *Etlingera littoralis* stems were purchased from wet market Bandar Riyal, Kota Samarahan. Then, the outer part of the stem was removed to get the cores of the young stems (Tepus). The young stems were cut into small pieces and rinsed with distilled water. Then, shade dried the samples (as shown in Figure 3.2). The samples were shade dried instead of using oven for drying (Chew, 2012). This is because, drying using oven may destroy plant chemical component (Azwanida, 2015). The samples were shade dried for about one week. After drying process, samples were ground in a blender to get dry powder. The dried powder of samples were stored in air tight bottles for future analysis. The powder samples were used instead of fresh samples because it will produce more homogenized and smaller particles that leading to better surface contact with extraction solvent (Azwanida, 2015).

Figure 3.2: Dried *Etlingera littoralis* cores of young stems.

3.2 Chemicals and reagents

The solvents used were methanol, ethanol and acetone from Merck (Germany). For dilution of the stock extract 10% dimethyl sulfoxide (DMSO) was used. Besides, the Muller-Hinton agar (Oxoid), Muller-Hinton broth (Himedia), nutrient agar (Merck) and gentamicin (Oxoid) were used.

3.3 Preparation of extracts

The ratio of 1:10 was used for the preparation of samples extract, where ten grams of dried powder of plant materials was weighed and extracted with 100 ml of different organic solvents in a conical flask (Parekh *et al.*, 2005). The organic solvents used were methanol, ethanol and acetone. Then, maceration technique was used by kept the soaking plant materials with solvent on the rotary shaker at 120 rpm for three days at room temperature to soften and break the plant cell wall which can release the soluble phytochemical (Azwanida, 2015).

After three days, the plant extracts were separated from the plant materials by filtration using Whatman filter paper (No.1). Then, the solvents were removed by evaporation using rotary evaporator (Buchi Rotavapor R-200) at 40-45°C to obtain the crude residue (Zaidan *et al.*, 2005). The crude extracts were weighed and the extracts were reconstitute in 10% dimethyl sulfoxide (DMSO). The stock extracts were diluted in DMSO in the two-fold serial dilution method in different concentration (50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml) and kept it for further studies.

3.4 Preparation of test bacteria

For the antibacterial screening, four bacterial species that are human bacterial pathogens were studied. The Gram-positive bacteria that have been used were *Staphylococcus aureus* and *Bacillus cereus.* The Gram-negative bacteria were *Escherichia coli* and *Pseudomonas aeruginosa.* All the bacteria strains were cultured and grown onto separate nutrient agar (NA) plates and it was incubated for 18-24 hours at 37°C. A single colony on each of the bacteria cultures were then subculture in 5 ml Muller-Hinton broth (MHB) and incubated it overnight at 37°C. This actively grown bacterial suspension that required for the antibacterial test was then adjusted to obtain turbidity comparable to 0.5 McFarland standard, (1.0×10^8 CFU/ml) by using spectrophotometer (Zaidan *et al.*, 2005).

3.5 Screening for antibacterial activity

The screening of antibacterial activity of the extracts was determined by agar well diffusion method (Jeyaseelan *et al.*, 2012). The suspensions of each tested bacteria (*S. aureus, B. cereus, P. euroginosa* and *E. coli*) was spread evenly on the surface of the Muller-Hinton agar (MHA) plate by using sterile cotton swab and allowed to dry at room temperature for about 30 minutes.

When the plates had been aseptically dried, 6 mm diameter wells were punched into each of these agar plates by using a sterile Pasteur pipette. There were six wells in each plate which include four different concentration of extracts (50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml), one negative control and one positive control. Approximately 10% DMSO and gentamicin were used as negative and positive controls respectively. The wells were filled with 50 µl of each concentration of plant extracts and also the positive control.

While 25 µl was for gentamicin. The plates were incubated at 37°C for 18-24 hours for all tested bacterial culture.

After incubation overnight, the antibacterial activity was evaluated by measuring the diameter of circular inhibition zones around the well. The diameter zones of inhibition produced by the extracts were compared to those produced by the negative control (10% DMSO) and the positive control (gentamicin). For each extract three replicate trials were conducted against each organism to get the average or mean diameter (mm) of each zones. The data were expressed as mean $\pm$ standard deviation.