UNIVERSITI PUTRA MALAYSIA

ANTIBACTERIAL AND CYTOTOXIC ACTIVITIES FROM SOME SELECTED PLANTS

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DEDICATION

This dedication goes to my loving mummy and daddy, May Fong, May Yen, May Wen, May Chen, Jong Chee, Jong Wei, Aunty Kwee, Uncle Tail, Pui Van, Pui Sie, Pui Cheng, Kheng Siong and Farn Sheng. Thanks for the love and support.

Love you all.

"You cannot catch a fish
Unless you put a line in the water

"You cannot reach your goals
Unless you actually do something"

ABSTRACT

Six plant extracts of four different species were studied for their antibacterial and cytotoxic activities. Methanol extract of leaf and stem from (*Phyllathus amarus*, *Senna alata*, *Cymbopogon nardus* and *Azadirachta excelsa*) were screened for antibacterial activity against *Vibrio parahaemolyticus*, *Escherichia coli, Listeria monocytogenes* and *Pseudomonas aeruginosa.* The antibacterial activity was determined using disc diffusion method. Most of the extracts showed relatively potential antibacterial activity against all of the tested microorganisms with the diameter of inhibition zones ranging between 7.0 mm to 17.9 mm with the concentration of 1.9 mg/ml to 1000 mg/ml. All the plants studied, except the stem of *Azadirachta excelsa*, was active against the tested microorganisms and the stem of *Senna alata* was found to be selective against *Vibrio parahaemolyticus*. The most active plant extracts was *Phyllathus amarus* while the least active was *Azadirachta excelsa* (leaf) against all four strains of bacterial. The cytotoxic activities of six extracts were determined using *Artemia salina* and tested at three concentrations: 10, 100 and 1000 µg/ml. All the extracts shown cytotoxic activity (LC_{50}<1000 µg/ml) except for the leaf of *Senna alata* (LC_{50} = 1173.0). The methanolic extract leaf of *Phyllathus amarus* was the most active (LC_{50} = 203.1) while with species moderate toxicity were the stem extracts of *Senna alata* (LC_{50} = 415.5) and *Azadirachta excelsa* (LC_{50} = 573.4). *Cymbopogon nardus* (LC_{50} = 680.8), the leaf extracts of *Azadirachta excelsa* (LC_{50} =1000.0) and *Senna alata* (LC_{50} =1173.0) were considered weak toxicity. The results obtained showed that the plants have potential antibacterial properties compare to cytotoxic properties. This can be selected for further investigation to determine its therapeutic potential as antibacterial agent.

ABSTRAK

Enam ekstrak tumbuhan dari empat spesies yang berlainan telah dikaji untuk mengetahui aktiviti antibakteria and sitotoksik. Ekstrak metanol bahagian daun dan batang dari beberapa tumbuhan (*Phyllanthus amarus*, *Senna alata*, *Cymbopogon nardus* and *Azadirachta excelsa*) telah dikaji bagi aktiviti antibakteria dengan *Vibrio parahaemolyticus*, *Escherichia coli, Listeria monocytogenes* dan *Pseudomonas aeruginosa*. Aktiviti antibakteria ditentukan dengan menggunakan kaedah disk penyerapan. Kebanyakan ekstrak menunjukkan potensi untuk aktiviti antibakteria terhadap semua organisma yang dikaji dengan diameter zon penghalangan merangkumi dari 7.0 mm kepada 17.9 mm dengan menggunakan kepekatan 1.9 mg/ml sehingga 1000 mg/ml. Semua tumbuhan yang dikaji, kecuali ektrak batang dari *Azadirachta excelsa*, adalah aktif terhadap kesemua mikroorganisma yang dikaji dan ekstrak batang dari *Senna alata* adalah memilih terhadap *Vibrio parahaemolyticus*. Ekstrak tumbuhan yang paling aktif adalah *Phyllanthus amarus* manakala ekstrak tumbuhan yang paling kurang aktif adalah *Azadirachta excelsa* (daun) tehadap keempat-empat strains bakteria. Aktiviti sitotoksik enam ektrak tumbuhan dikaji dengan menggunakan tiga kepekatan: 10, 100, 1000 µg/ml. Semua ekstrak menunjukkan aktiviti sitotoxik (LC_{50} < 1000) kecuali ektraks daun dari *Senna alata* (LC_{50} = 1173.0). Ekstrak metanol dari daun *Phyllanthus amarus* adalah paling aktif (LC_{50} = 203.1) manakala spesies yang mempunyai kesederhana toksik dari ekstrak batang dari *Senna alata* (LC_{50} = 415.5) dan *Azadirachta excelsa* (LC_{50} = 573.4). *Cymbopogon nardus* (LC_{50} = 680.8), daun ekstrak *Azadirachta excelsa* (LC_{50} = 1000.0) dan *Senna alata* (LC_{50} = 1173.0) adalah toksiksiti lemah. Keputusan yang diperolehi menunjukkan tumbuhan tersebut dianggap mempunyai potensi sifat antibacterial berbanding dengan sifat sitotoksik. Ini dapat dipilih untuk

penyelidikan yang selanjutnya untuk menentukan potensi therapeutiknya sebagai agen antibakteria.

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APPROVAL SHEET

I certify that this research project report entitled "Antibacterial and Cytotoxic Activities from Some Selected Plants" has been examined and approved as a partial fulfillment of the requirement for the degree of Bachelor of Bioindustry Science in the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus.

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LIST OF ABBREVIATIONS

ml : mililitre

mm : milimetre

μg/ml : microgram per mililitre

% : percentage

LC_{50} : Lethal concentration

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CHAPTER I

INTRODUCTION

1.1 Background

Throughout the history of mankind, a large number of the earth population especially those from undeveloped countries rely on medicinal plants to meet their health care needs. In recent years, traditionally used medicinal plants have received attention of the pharmaceutical and scientific communities, especially from the west to develop new effective drugs in order to support the increasing population of the world (Cordell, 2002). In addition, scientific interest in medicinal plants has burgeoned due to increased efficiency of new plant-derived drugs and growing interest in natural products.

Medicinal plants are defined as those which produce one or more active constituents capable of preventing or curing an illness (Juriyati *et al.*, 1995). In fact, plant produces a diverse range of bioactive molecules, making them a rich source of different types of medicines. Up to 80 % of the worlds' population totally depends on plant materials as their source of primary health care (Cordell, 2002). There are 400,000-500,000 plants species are believed to exist, but only small percentage have yet to be discovered (Tringali, 2001). This means that there is a need to add more plants species to boost the image and importance of medicinal plants. Hence, rapid disappearance of tropical forests has meant that it is essential to have access to methods which lead to the rapid isolation and identification of bioactive natural products.

Malaysia has been identified as one of the world's megadiversity areas with extremely rich biodiversity. The huge diversity of the Malaysian flora means that we can expect well diverse chemicals structure from their metabolites. The well diversity of flora also means that a wide variety of secondary metabolites can be produced. Thus the probability to find a bioactive compound is enormous.

There is no doubt that plants are a good source of biologically active natural products. Plants contain a great variety of compounds, thus extraction and separating of individual compounds from extraction is frequently arduous. In order to discover bioactive compounds, it is essential to have access to simple biological tests to locate required activities.

1.2 Objectives

The objectives of this research were to:

1. Evaluate antibacterial properties of *Phyllanthus amarus*, *Cymbopogon nardus*, *Senna alata* and *Azadirachta excelsa* against four strains of bacteria, *Vibrio parahaemolyticus*, *Escherichia coli*, *Listeria monocytogenes* and *Pseudomonas aeruginosa*.

2. Evaluate cytotoxic properties of these plants on brine shrimp lethality test to obtain a preliminary indication of their potential biological activity.

CHAPTER II

LITERATURE REVIEW

2.0 Medicinal Plants

According to Samy *et al*., (2005), there is around 8,100 species plant species in Malaysian rain forest with 10 % of them reported to have some medicinal values. In addition, the total rain forest area in Malaysia is about 19.12 million hectares occupying some 58.1 % of the land area of the country. There is a wide potential to discover the drugs that are beneficial to human health.

Plants, especially medicinal plants, offer a vast resource of novel natural compounds often with exciting activities and biological properties. It has a valuable source of new and biologically active molecules. It is important to have the necessary tools at hand. These include suitable biological assays and chemical screening methods.

Medicinal plants are widely used by all section of people as folk remedies or in different indigenous system of medicine or indirectly in the pharmaceutical preparations of modern medicines (Srivivasan *et al.,* 2001). According to Samy *et al*., (2005), it is estimated that some 119 active compounds isolated from higher plants are used in modern medicine. Out of these, 74 % of them are derived from plants that have some related use in traditional herbal medicine. For example, the papaya, *Carica papaya* which was traditionally used to treat eczema and warts. The enzyme papain is now used to heal wounds and the papaya extract to treat *Candida albicans*.

2.1 The Role of Natural Product in Drugs Discovery

Historically, natural products play a very vital role in the discovery of new and novel drugs especially plant derived drugs. Many traditional drugs originate from plants sources and some of the most effective drugs are plant based. For instance, aspirin derived from bark of willow, quinine derived from the bark of cinchona, and morphine derived from the O*pium poppy*. Besides that, medicines derived from plants are easily available and cheaper than modern medicine (Mathabe *et al*., 2006).

According to Taylor 2000, there are 120 distinct chemical derived from plants are considered as important drugs currently being used in one or more countries in the world. Several of the drugs sold today are simple synthetic modifications or copies of the naturally obtained substances. For example, taxol which approved in 1992 and semi-synthetic in 1995 is a natural product derived from the Pacific yew tree *Taxus brevifolia*, which is used for the treatment of ovarian and breast cancer. The successes of natural products have proved that it can be used against the most life threaten diseases like AIDS, SARS, infectious diseases etc.

Many natural products were reported in literature to have antimicrobials properties which are effective in treatment of infectious diseases such as diarrhea (Mathabe *et al*., 2006), antibacterial and wound healing (Ming *et al*., 2005) and treatment of cutaneous conditions (Lopez *et al*., 2001). Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic microbials. According to Nair *et al*., (2005), plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. Subsequently, simple bioassay should be developed for screening plant extracts to detect plant compounds with relevant biological activities which may be used as a guide for fractioning plant extracts.

2.2 Antibacterial Agents from Natural Products

Historically, plants have provided a source of inspiration for novel drug compounds, where the plant derived medicines have made large contributions to human health and well-being. In addition, the primary benefits of using plants derived medicines have proved that they are relatively safer than synthetic alternatives, conducing profound therapeutic benefits and more affordable treatment (Iwu *et al.*, 1999).

The effect of plants extract on bacteria has been studied by a very large number of researchers in different area of the world. The vast number and variety of chemotherapeutic agents isolated from microbial natural products and used to treat bacterial infections have greatly contributed to the improvement of human health during the past century. Infection disease is number one cause of death accounting for approximately one-half of all deaths in tropical country (Iwu *et al.*, 1999). This is alarming given that we must eliminate infectious disease so soon as possible in order to prevent from harming of human lives. Therefore, the emergence of pathogenic microbes with increased resistance to established antibiotics provides a major incentive for the discovery of new antimicrobial agents. Antimicrobial screening of plant extracts and phytochemicals would represents starting point for antimicrobial drug discovery.

Sohn *et al*., (2004) strongly suggested that natural products are the major source of important antimicrobial agents. It is expected that screening of plant extracts against wide variety of test microorganisms will be helpful in obtaining broad spectrum herbal formulation as well as new antimicrobial substances. In addition, there is a need to document the valuable knowledge on medicinal plants because of rapid disappearance of forest habitats in Malaysia. Besides that, urgent attention has to be paid where there is around 350,000 plant species on earth which is a large percentage still have not been investigated (Hostettmann and Marston, 2002). It is imperative that a screening program must be implemented and administered accordingly with the ultimate goal of discovering new compounds from the Malaysian forest.

2.3 Cytotoxicity in Medicinal Plants

Brine shrimp lethality test is a general bioassay of detecting a broad spectrum of bioactivity present in crude extract. Due to its commercial availability, the eggs of brine shrimp, *Artemia salina* (Leach), is an invertebrate widely used in toxicological applications and research. According to McLauglin *et al*., (1998), brine shrimp lethality test had been used as prescreen for over 300 novel antitumour and pesticidal natural products. Thus it is possible to detect potent extracts using this bioassay rather than more tedious and expensive *in vitro* and *in vivo* antitumour assay. They provide a quick, inexpensive, simplicity and utilizes small amount of test material (Parra *et al*., 2001).

Since its introduction in 1982, this *in vivo* lethality test has been successively employed for bioassay-guide fractionation of active cytotoxic such as twenty plants samples from eleven plant families in Nigerian (Ajaiyeoba *et al*., 2006), *Kaempferia galangal* (Othman *et al*., 2006) and aqueous extract of hundreds and eighteen of medicinal plants in India (Krishnaraju *et al*., 2006), five members of Thai medicinal plants in the family *Meliaceae* (Pisutthanan *et al*., 2004), eighteen different extracts from five *Annona* species from Brazil (Pimenta *et al*., 2003), *Commiphora mukul* and *Piper longum* (Padmaja *et al*., 2002). From the research, almost promising results can be obtained and found to be consistent with existing phytochemical knowledge of these plants as a source of cytotoxic and antitumour compounds. In few cases, complete analysis of the cytotoxicity of several plant parts allows researchers to understand the location of cytotoxic substances (Krishnaraju *et al*., 2006).

It would seem logical to prescreen plant extracts with general bioassay, which is brine shrimp lethality test in order to predict their toxicity. Extracts must be screened for biological activity, then the active extracts selected, fractionations directed with bioassays and then the bioactive compounds identified (Mclaughlin *et al*., 1998).

2.4 Biologies Activites of Medicinal Plants

2.4.1 Phyllanthus amarus Scum. & Thonn. (Euphorbiaceae)

Figure 1: The leaves and fruits of *Phyllanthus amarus*

Phyllanthus amarus comes from family Euphorbiaceae. *Phyllanthus amarus* is native to tropical America, and spread to the tropical regions of the world (Samy *et al*., 2005). It is a small, erect, annual herb, 30 to 40 cm in height with small ovate to elliptic leaves, distichously arranged on branches resembling a pinnate leaf with small unisex flowers and spherical 6-lobed capsules containing ribbed seeds (Figure 1). *Phyllanthus amarus* occurs as a weed throughout the tropical region.

The genus *Phyllanthus* contains over 600 species of shrubs, trees, and annual or biennial herbs distributed throughout the tropical and subtropical regions of both hemispheres (Murugaiyah *et al*., 2006). Its main uses are as effective remedy to eliminate gallstones and kidney stones (Samy *et al*., 2005). It can used to treat colds, flu, liver diseases and disorders including anemia, and liver cancer; as well as for its diuretic, pain-relieving, digestive stimulant, antispasmodic, fever reducing, and cellular protective properties in many other conditions (Kassuya *et al*., 2006). There are several biological activities have been reported, including antihyperglycaemic (Husen *et al*., 2004) and antifungal activities (Hoffman *et al*., 2004). It is important especially in treating hyperlipidemia and obesity which are closely associated with hypertension and stroke. *Phyllanthus amarus* also has been shown to have antibacterial activity (Klouek *et al*., 2005) and widely employed for diabetes (Adenele *et al*., 2006).

According to Rajeshkumar *et al*., (2002), *Phyllanthus. amarus* extract has been used as an antiviral agent to inhibit DNA polymerase of hepatitis B virus and other related hepatitis viruses. Besides that, aqueous extracts also exhibited potent carcinogenic activity while methanolic extracts of *P. amarus* also significantly inhibited gastric lesion, mortality, increased stomach weight, ulcer index and intraluminal bleeding were also reduced significantly (Raphaeal *et al*., 2003).

In Malaysia, *P. amarus* is used for treating diarrhoea, kidney ailments, gonorrhea and syphilis. It is especially recommended for kidney ailments where a decoction of the young leaves and root is taken. Hence, the young leaves are used for treating coughs, as a tonic to treat women after childbirth or a miscarriage. It is boiled with goat's milk and given to children and babies to treat jauntice (Samy *et al*., 2005)

2.4.2 *Senna alata* Roxburgh (Leguminosae)

Figure 2: Leaves of *Senna alata*

Senna alata comes from famili Leguminosae. *Senna alata* known as emperor's candlesticks or christmas candles is a loaf large shrubby legume growing up to four metres in height (Figure 2). Locally known as 'suruok' or 'pringang' in Bidayuh, 'rugan' or 'serugan' in Iban, 'tarum' in Penan and 'gelenggeng' or 'daun solok' in Malay. According to Ahmad *et al*., (2003), pounded leaves are used externally for stomach aches while young leaves are crushed and applied as a paste for skin infections such as ringworm and scabies. *Senna alata* has been reported that contained antibacterial and antifungal activities (Owoyale *et al*., 2005, Phongpaichit *et al*., 2004, Awal *et al*., 2004, Ahmad and Ismail, 2003). For the assessment of cytotoxic property by using brine shrimp lethality test were reported (Awal *et al*., 2004), where seed extract was more toxic compare to leaf extract. Owayale *et al*., 2005 reported that *S. alata* strongly inhibited bacterial and fungal activity and phytochemical its component was investigated. Although a wide range of literature reported that there are many antimicrobial active compounds presence in *S. alata*, but Adedayo *et al.*, 2002 had proved that the mechanism antibacterial activity of the *S. alata* plant extract involved potassium ion and protein leakage.

2.4.3 *Cymbopogon nardus* Rendle (Poacea)

Figure 3: *Cymbopogon nardus*

Cymbopogon nardus locally known as 'Serai Wangi' while commercially known as Citronella oil comes from family Poacea. According to Samy et al., (2005), *Cymbopogon nardus* is tall, clumped perennial grass growing to a height of 1.5 m. The leaves about 2.5 m wide grow to about 1 m long, and glabrous with sharp leaf margins (Figure 3). Traditionally, the leaves of *C. nardus* are used for their fragrance, insect repellent, relieve rheumatism, fever and for digestive problems. Besides that, many biological effects also have been carried out in Malaysia like FRIM (Forest Research Institute Malaysia) where citronellal oils in general exhibited a better antifungal inhibitory activity than antibacterial. They can be used in health products such as shampoo, insect repellent, soaps and cosmetics.

Previous studies have revealed that *C. nardus* exhibited antibacterial and antifungal activities. The antifungal assay using the vapor-agar contact method showed that the crude essential oil suppressed the growth of the several species of fungus (Nakahara *et al*., 2003). Brasileiro *et al*., 2006 reported that *C. nardus* exhibited antibacterial activities and classified as toxic using brine shrimp lethality (LD_{50} <1000ppm).

2.4.4 *Azadirachta excelsa* (Jack) M. Jacobs (Meliaceae)

Figure 4: *Azadirachta excelsa*

Azadirachta species from the Meliaceae family have attracted a great deal of attention due to their bioactive compounds. In Sarawak, one species is known for its timber properties (Figure 4). The species *Azadirachta excelsa* is locally known as 'Sentang' or Sarawak known as 'Ranggu'. Besides having timber properties, the young shoots and flowers can be consumed as vegetables while the seed contain Azadirachta, which is used as insecticide (Joker, 2000). They have a great deal of attention due to their medicinal values. Many biological effects have been reported that extracts of plants in the genus *Azadirachta*, with estrogenic and antipyretic, feeding inhibitory, insect growth inhibitory, antibacterial and antifungal, antinflammatory, antiulcer, antimalaria, cytotoxic and antiHIV (Cui *et al*., 1998).

Table 1: Ethnobatanical data on medicinal species obtained in UPMKB

Botanical names and family	Malaysian name	Traditional usage
Azadirachta excelsa, Meliaceae	Sentang	Timer production, pesticidal properties, young leaves and fresh flower can be used as food, seed use as natural insecticide
Senna alata, Leguminosae	Gelenggeng or daun solok	Treat many types of skin disorder such as ringworm, white spot and itchiness, curing constipation , roots is for diarrhea
Phyllanthus amarus, Euphorbiaceae	Dukung anak	Eliminate gallstones and kidney stones, anti-viral against Hepatitis B, antibacterial and antifungal activity.
Cymbopogon nardus, Poaceae	Serai wangi	Used as mosquito repellent, household fumigant, fragrance agent in food commodities, soaps and cosmetics.

2.5 Phytochemical on Medicinal Plants

The phytochemical research based on ethnopharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants. Plants from family Euphorbiaceae have been investigated intensively, motivated initially by the lignans such as phyllathin, hypophyllanthin, flavanoids, quercetin, astragalin, ellagitannins and hydrolysable tannins are proved to be present in this plant (Rajeshkumar *et al*., 2002). Some of these compounds are found to indicate that the plant may be more effective in the condition of liver tumours and against experimental carcinogenesis.

Many reports have shown that some *Senna* species contain antimicrobial substances, particularly *Senna alata* and they are recommended for primary health care in Thailand to treat ringworm and skin diseases (Phongpaichit *et al*., 2004). It has been observed that antimicrobial activity of the plants is associated with the presence of some chemical compounds such as phenols, tannins, saponins, alkaloids, steroids, flavonoids and carbohydrates (Owoyale *et al*., 2005). Previously, it has been reported that to contain anthraquinone, which is the principle laxative constituent of many plants used as purgative. Similarly, phytochemical screening has proved that flavonoid glycoside to be the most active by the chemical test.

Major components in *Cymbopogon nardus* are citronella, citronella, geranyl acetate, geraniol, trans-citral and cis-citral (Nakahara *et al*., 2003). It has been reported that citronella oil exhibited antibacterial and antifungal activities by using disc diffusion methods. Besides that, *C. nardus* also exhibited antiviral activity against measles virus and indicated the presence of antiviral substances only active against enveloped viruses (Aini *et al.*, 2006).

Many biological effects have been reported for extracts of plants in the genus *Azadirachta,* which are estragenic and antipyretic, feeding inhibitory, insect growth inhibitory, antibacterial and antifungal, antimalaria, anti-inflammatory, cytotoxic, and antiHIV being the most frequently cited. The essential oil present in the genus of *Azadirachta* has been evaluated by using gas chromatography and gas chromatography-mass spectrometry. Kurose and Yatagai, 2005 reported that *A. excelsa* contained oleic acid (31.3 %), hexadecanoic acid (14.2 %), octadecanoic acid (13.0 %), 4-octylphenal (9.7 %), and 0-methyloximedecanal (6.8 %) as the main constituents. In the other hand, *A. excelsa* were broadly cytotoxic against a panel human cancer cell line (Cui *et al*., 1997). It is proved that there are two constituents were novel, namely 2, 3-dihydronimbolide and 3-deoxymethylnimbidate as cytotoxic constituents.

CHAPTER 3

METHODOLOGY

3.1 Plants collections

Fresh leaves and stems of *A. excelsa* and *S. alata* were collected from Taman Botani, UPMKB; whole plants (leaf and stem) of *P. amarus* and *Cymbopogon nardus* (Table 1) were collected from TPU (Taman Pertanian Unit), UPMKB. They were collected with the help of the staffs from Department of Forestry Science and identified and authenticated by Prof Madya Dr Rajan and Prof Madya Dr Joseph Bong. Collected plants materials were air-dried at room temperature and grind with an electric grinder into fine powders which were stored into airtight containers at room temperature.

3.2 Plants Extraction

Dried powdered plants materials were extracted with methanol at room temperature for 3 to 4 days. At the end of extraction each extract was passed through Whatman filter paper No. 1 (Whatman Ltd, England). The filtrate was concentrated on a rotary evaporator (Buchi Rotavapor R-200) under vacuum at 60 °C for further use. The yields from the methanolic extracts were weighted and recorded (Table 2).

Table 2: Extraction of crude extracts

Plant species	Part used	Wet weight (g)	Dry weight (g)	Extract (g)	Yield (%)
Azadirachta excelsa	Leaves	364.17	131.00	11.40	8.70
	Stem	109.50	52.00	5.70	10.96
Senna alata	Leaves	821.90	425.10	19.86	4.67
	Stem	109.50	52.00	5.70	15.64
Phyllanthus amarus	Whole part	435.00	250.00	41.27	16.51
Cymbopogon nardus	Leaves	1256.00	586.00	127.76	21.80

3.3 Antibacterial Assay

3.31 Test Microorganisms

Four bacterial were employed as test organism. These include: *Pseudomonas aeruginosa, Escherichia coli, Listeria monocytoges and Vibrio parahaemolyticus* which were obtained as fresh pure cultures from UPM, Serdang.

3.3.2 Preparation of stab cultures

Bacteria can be stored up to 1 year as stabs in soft agar. Nutrient agar was prepared and autoclaved, allowed it to cool and poured into a screw cap vial under sterile conditions, and then left to solidify. Using a sterile straight loop, single colony was picked from a freshly grown plate and stabed it deep down into the agar. The vial was incubated overnight at 37 °C by leaving the cap slightly loose prior to storage. After that, the vials were then kept in refrigerator for future usage.

3.3.3 Cultures and Inoculum

Bacterial strains used were maintained on nutrient agar (Merck, 1.05450.0500) and subcultures were freshly prepared before use. Bacterial were prepared by transferring one colony into a tube containing 5 ml nutrient broth (Merck, 1.5443.0000) and grown overnight at 37 °C in incubator.

3.3.4 Preparation of Medium

Nutrient agar (Merck, 1.05450.0500) was prepared in 20 ml amounts, sterilized by autoclaving, allowed to cool and then poured into a sterile Petri dish and allowed to solidify for 1 hour. Each nutrient agar was spread using hockey stick with broth cultures of each of the test organisms.

3.3.5 Evaluation of Extracts for Antibacterial Activities

The antibacterial activities of the extracts were determined using Whatman filter paper No. 1 (Whatman Ltd, England) was punched with 6 mm diameter. The filter paper was impregnated with 10 μl of a solution prepared with 1000 mg of extract in 1ml of methanol, (corresponding to 500, 250, 125 mg/mL of crude plant extract) and allowed to dry at room temperature. The impregnated filter papers were placed on the fresh spread plates containing the testing bacterial and incubated overnight before the resulting zones of inhibition were observed and recorded. The bacterial activity was expressed as the mean of diameter of the inhibition zones (mm) produced by the plant extracts (Appendices C). Generally, the more susceptible the organism the larger is the zone of inhibition. Each sample was done in triplicates. Ciprofloxacin was used as positive control while methanol as negative control. Tests were repeated thrice.

3.3.6 Preparation of Positive Control (Ciprofloxacin)

Ciprofloxacin was obtained from pharmarcy in Medan, Bintulu with the concentration of 500 mg in a pill form. To prepare the stock solution at the concentration of 500 µg/ml, the pill was crushed to powder form by using mortar and pestle. Subsequently, the powder was mixed with 1000 ml of distilled water. With the required concentration of ciprofloxacin, it was pipetted into Whatman filter paper No. 1 (Whatman Ltd, England). The filter paper with the diameter 6mm was impregnated with 10 µl of a solution and placed on the plates that had been spread with testing bacterial. It was allowed to incubate overnight in the incubator and the resulting zones of inhibition were observed and recorded. Each sample was done in triplicate and served as control.

3.3.7 Statistical Analysis

All the experiments were performed in triplicates. The mean and standard deviation of these experiments were determined (Appendices A). The data were analysed for the effects of bacteria within extracts on inhibitory zones using general linear model (GLM) procedure of the NCSS statiscal package (Table 9). Differences were considered significant at $P<0.05$.

3.4 Cytotoxicity Assays

3.4.1 Brine shrimp lethality test

Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of methanol extracts of plant materials. It was carried out with some slight modifications to Othman *et al.*, (2006). The eggs of brine shrimp (*Artemia salina*) and salt water were readily obtained from UPMKB Hatchery Unit. Eggs (1 g) were placed in container with 1000 ml of salt water while light was on one side of the container. The eggs were left for 48 hours to hatch. The test was repeated thrice. Salt water was used as the control excluding the test sample and solvent.

Then 20 mg of extracts was accurately measured and dissolved in 2 ml methanol to get a concentration 10 mg of stock solution. From the stock solution, volumes of 500, 50, 5 μl were transferred into the glass vials, and these corresponded to 1000, 100, and 10 $\mu g ml^{-1}$, respectively. The solvent were dried by putting in desiccator overnight. To each vial, 5 ml of brine were added, followed by 10 shrimps for 24 hours. After 24 hours, the vials were observed using a magnifying glass and the numbers of survivors in each vial were counted and recorded. The resulting data were transformed to the probit analysis for the determination of LC_{50} values for the extracts (Appendix B).

CHAPTER 4

RESULTS

4.1 Antibiotic Test

The positive control used was a broad spectrum antibiotic, Ciprofloxacin (FS 2722). The concentration of antibiotic was chose according to intermediate zone diameter from 16 to 20 cm (NCCLS, 1998). For *E. coli* and *Listeria monocytogenes*, the concentration of 0.05 µg/ml was used whereas concentration of 0.02 µg/ml for *P. auruginosa* and V*ibrio parahaemolyticus.*

Table 3: Inhibition zone of bacteria strains against various concentration of antibiotic

	Diameter of zone of inhibition (mm)			
	Concentration, µg/ml			
Test organisms	0.05	0.02	0.01	0.005
Escherichia coli	20.5	17.0	11.5	8.0
Listeria monocytogenes	20.0	19.5	11.5	7.0
Pseudomonas aeruginosa	10.0	18.0	14.0	21.5
Vibrio parahaemolyticus	26.0	18.0	-	-

Source: National Committee on Clinical Laboratory Standard (NCCLS), 1998

4.2 Antibacterial assay

Preliminary evaluation of antibacterial activity of six samples of crude extracts exhibited antibacterial properties except for the stem of *A. excelsa*, which was unable to inhibit any of the bacterial strains tested. This indicates that the presence of potent antibacterial activity in these plants.

Among six samples of plant extracts showed antibacterial activity with the inhibitory zones ranging from 7.0 mm and 17.9 mm. It could be seen that *P. amarus* have a wide range of inhibition zone ranging from 7.0 mm to 17.9 mm (Table 4) followed by *C. nardus* 6.9 mm to 14.2 mm (Table 5), *S. alata* leaf ranging from 7.6 mm to 13.8 mm (Table 6), *A. excelsa* leaf ranging 7.0 mm to 9.8 mm (Table 7) while activity of S. *alata* (stem) was selective against *V. parahaemolyticus* ranging from 7.6 mm to 11.0 mm (Table 8). But in the other hand, *A. excelsa* (stem) was inactive against four bacterial strains.

The methanol extracts from *Phyllanthus amarus*, *Cymbopogon nardus*, *Senna alata* and *Azadirachta excelsa* showed significant differences ($P<0.05$) in antibacterial activity. Of all the plants studied, the most active extract was *P. amarus* due to the ability to inhibit bacterial growth at the lowest concentration of 3.9 mg/ml in three different strains except for *V. parahaemolyticus* which was only inhibited at 31.3 mg/ml or higher. There were significant differences among all the extracts at the lowest concentration against all bacteria tested except for *Vibrio parahaemolyticus* (Table 9). The inhibitory zone at the lowest concentration of 3.9 mg/ml was at 8.7 mm for *E. coli*, 9.3 mm for *L. monocytogenes* and 8.4 mm for *P. aeruginosa* while at 31.3 mg/ml against *V. parahaemolyticus* was 7.0 mm.

Meanwhile for *Cymbopogon nardus,* the lowest concentration at which activity against *L. monocytogenes* occurred was at 7.8 mg/ml, and at 15.7 mg/ml the extract was inhibitory to all four bacterial strains. *Cymbopogon nardus* showed no significant differences at the lowest concentration of 7.8 mg/ml with *P. amarus,* while *S. alata* and *A. excelsa* was both inactive against *Listeria monocytogenes.* Besides that, *Cymbopogon nardus* also showed significant differences at 15.7 mg/ml among all the plant extracts against *V. parahaemolyticus, E. coli* and *Pseudomonas aeruginosa*. The inhibitory zone at the lowest concentration of 7.8 mg/ml was at 8.4 mm against *Listeria monocytogenes*. The other three bacterial strains were inhibited at 15.7 mg/ml with diameters of 6.9 mm for *V. parahaemolyticus*, 8.6 mm for *E. coli* and 8.8 mm for *Pseudomonas aeruginosa.*

The leaf of *Senna alata*, the lowest concentration at which activity against *Vibrio parahaemolyticus* occurred was at 125 mg/ml and showed no significant differences to *P. amarus*, but was significantly different from *C. nardus* and *Azadirachta excelsa*. At the lowest concentration of 250 mg/ml, *S. alata* started to inhibit the growth of *E. coli* and with *Listeria monocytogenes.* The extract was significant different from all other extracts. The lowest concentration at which *S. alata* was inhibitory to *P. aeruginosa* was 500 mg/ml and showed significant different with *C. nardus* and *P. amarus* but no significant different with *Azadirachta excelsa*. The inhibitory zones recorded were 8.7 mm for *V. parahaemolyticus*, 8.2 mm for *L. monocytogenes*, 7.9 mm for *P. aeruginosa* and 7.6 mm for *Esherichia coli*. On the other hand, the stem extract of *S. alata* was selective against *V. parahaemolyticus* but unable to inhibit the other three bacterial strains tested. The lowest concentration at which it inhibits to growth of *V. parahaemolyticus* was 25 mg/ml with inhibitory zone of 7.6 mg/ml.

The leaf extract of *A. excelsa* was mildly active with the minimum inhibitory concentration of 250 mg/ml against *E. coli*, and 500 mg/ml against *V. parahaemolyticus*, *L. monocytogenes* and *Pseudomonas aeruginosa*. Among all the plant extracts, *A. excelsa* required higher concentration to inhibit the growth of tested bacteria. In addition, this plant was the least active among all the extract against tested bacteria. The inhibitory zones were 7.3 mm against *E. coli*, followed by 7.0 mm for *V. parahaemolyticus*, 7.8 mm for *L. monocytogenes* and 8.1 mm for *Pseudomonas aeruginosa*. As stated earlier, the stem extracts of *A. excelsa* was inactive against all four bacterial strains. There was no inhibition in growth of all bacterial strains tested with the negative control (methanol).

4.2.1 Extract of *Phyllanthus amarus*

Table 4: Inhibitory effects of *P. amarus* against four bacterial strains

Bacterial strains	Diameter of zone of inhibition (mm) Concentration, µg/ml									
	1000	500	250	125	62.5	31.3	15.7	7.8	3.9	1.9
Vibrio parahaemolyticus	10.4	9.5	8.1	7.9	7.1	7.0	0.0	0.0	0.0	0.0
Escherichia coli	16.0	15.1	13.6	12.4	12.2	11.1	11.3	10.3	8.7	0.0
Listeria monocytogenes	17.9	14.7	14.6	14.0	13.8	12.4	11.0	9.8	9.3	0.0
Pseudomonas aeruginosa	16.0	14.7	12.9	12.4	12.0	11.7	11.6	10.2	8.4	0.0

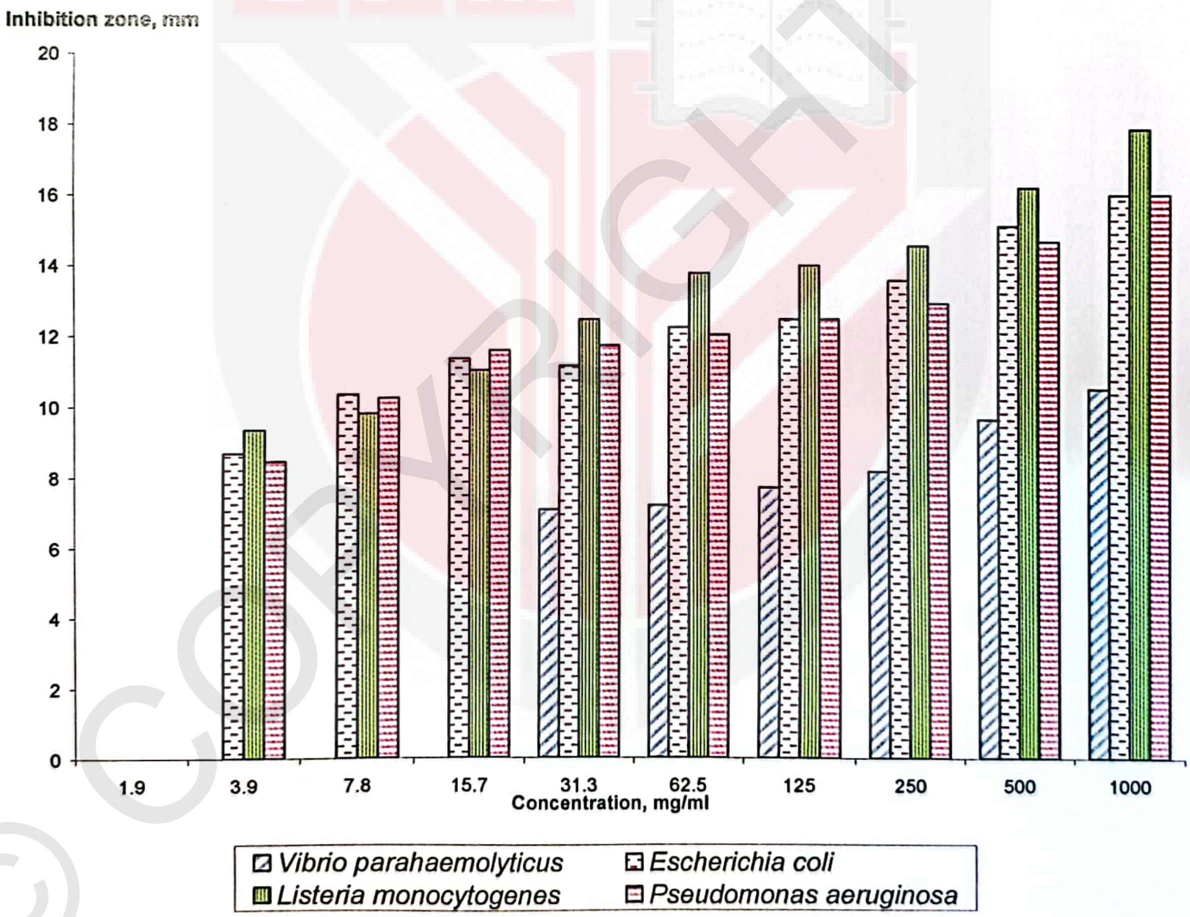

Figure 5: Inhibitory effects of *P. amarus* against four bacterial strains

4.2.2 Extracts of *Cymbopogon nardus*

Table 5: Inhibitory effects of *C. nardus* against four bacterial strains

Bacterial strains	Diameter of zone of inhibition (mm)								
	Concentration, µg/ml								
	1000	500	250	125	62.5	31.3	15.7	7.8	3.9
Vibrio parahaemolyticus	13.5	13.3	12.8	12.0	11.2	10.0	6.9	0.0	0.0
Escherichia coli	14.2	13.0	12.1	11.4	11.3	9.6	8.6	0.0	0.0
Listeria monocytogenes	14.1	12.9	12.7	12.3	10.3	10.5	9.6	8.4	0.0
Pseudomonas aeruginosa	13.1	12.6	12.4	11.6	11.5	11.1	8.8	0.0	0.0

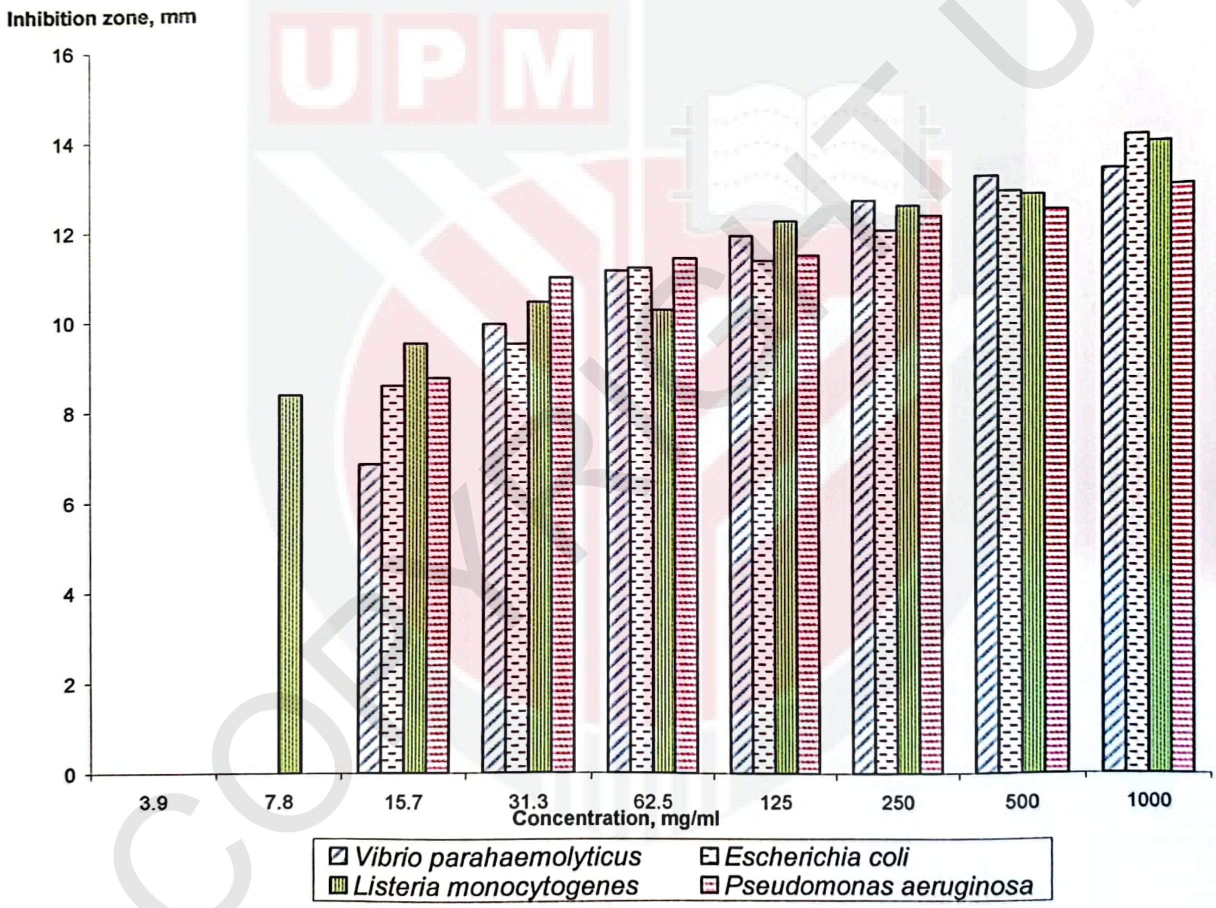

Figure 6: Inhibitory effects of *C. nardus* against four different bacterial strains

4.2.3 Extracts of *Senna alata* (leaf)

Table 6: Inhibitory effects of *S. alata* (leaf) against four bacterial strains

Bacterial strains	Diameter of zone of inhibition (mm)				
	Concentration, µg/ml				
	1000	500	250	125	62.5
Vibrio parahaemolyticus	13.8	12.1	10.6	8.7	0.0
Escherichia coli	9.0	7.6	0.0	0.0	0.0
Listeria monocytogenes	11.2	8.6	8.2	0.0	0.0
Pseudomonas aeruginosa	10.0	7.9	0.0	0.0	0.0

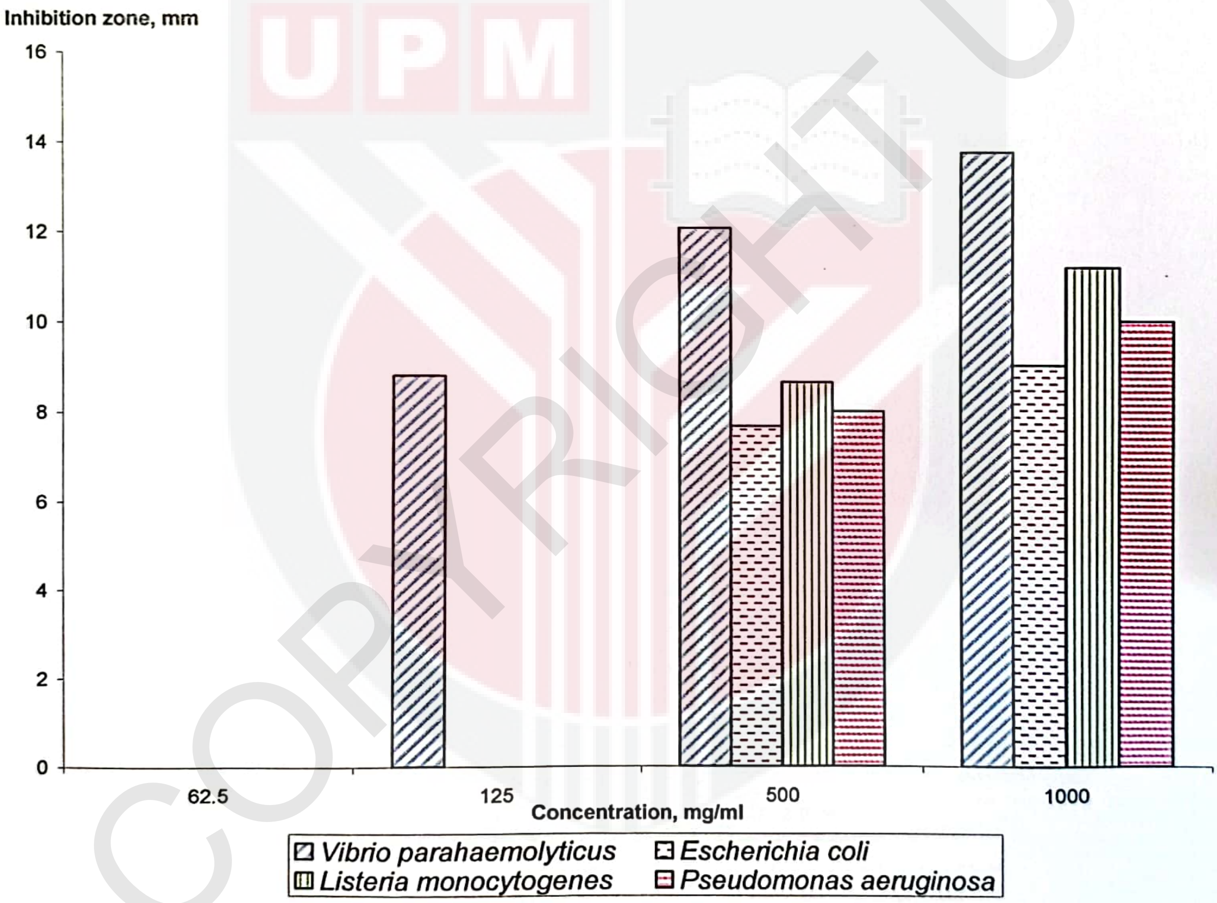

Figure 7: Inhibitory effects of *S. alata* (leaf) against four bacterial strains

4.2.4 Extract *Senna alata* (stem)

Table 7: Inhibitory effects of *S. alata* (stem) against four bacterial strains

Bacterial strains	Diameter of zone of inhibition (mm)			
	Concentration, µg/ml			
	100	50	25	12.5
Vibrio parahaemolyticus	11.0	8.6	7.6	0.0
Escherichia coli	0.0	0.0	0.0	0.0
Listeria monocytogenes	0.0	0.0	0.0	0.0
Pseudomonas aeruginosa	0.0	0.0	0.0	0.0

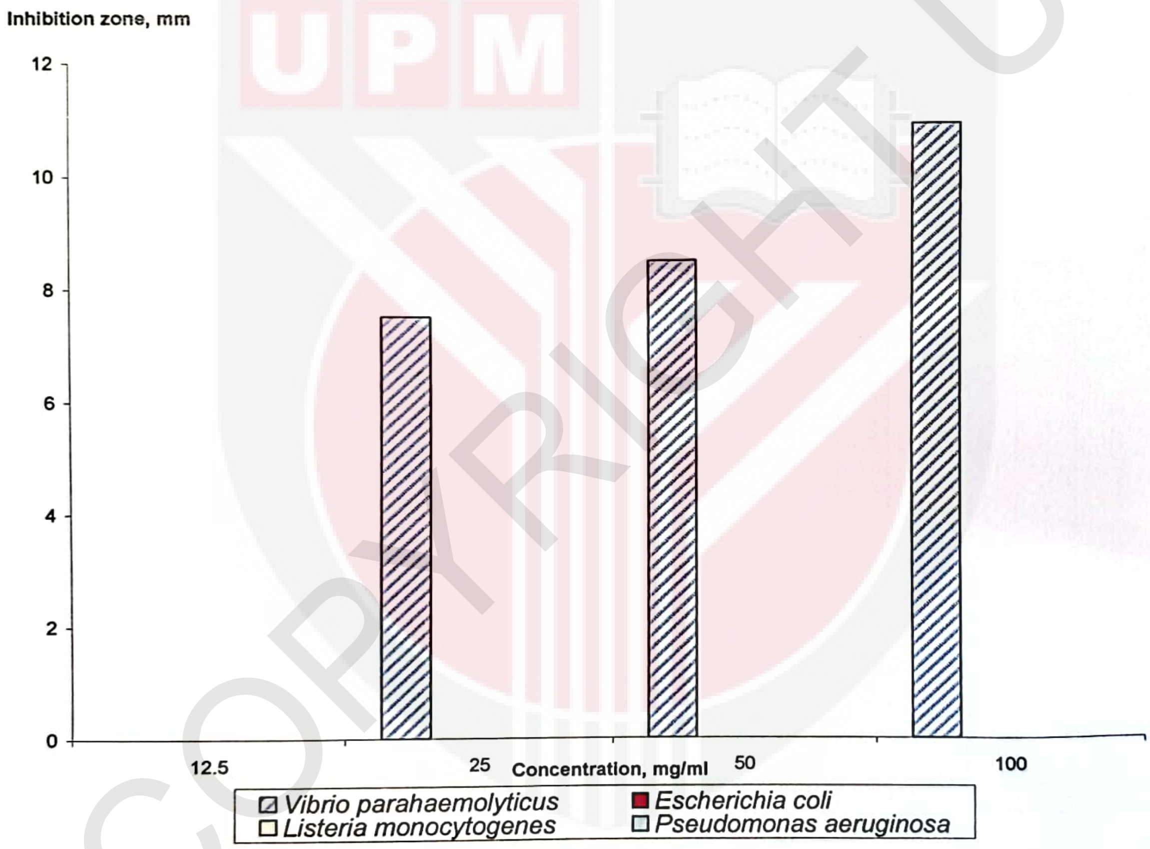

Figure 8: Inhibitory effect *S. alata* (stem) against four bacterial strains

4.2.5 Extract of *Azadirachta excelsa* (leaf)

Table 8: Inhibitory effects of *A. excelsa* (leaf) against four bacterial strains

Bacterial strains	Diameter of zone of inhibition (mm)			
	Concentration, µg/ml			
	1000	500	250	125
Vibrio parahaemolyticus	7.3	7.0	0.0	0.0
Escherichia coli	7.0	7.4	7.3	0.0
Listeria monocytogenes	8.1	7.8	0.0	0.0
Pseudomonas aeruginosa	9.8	8.1	0.0	0.0

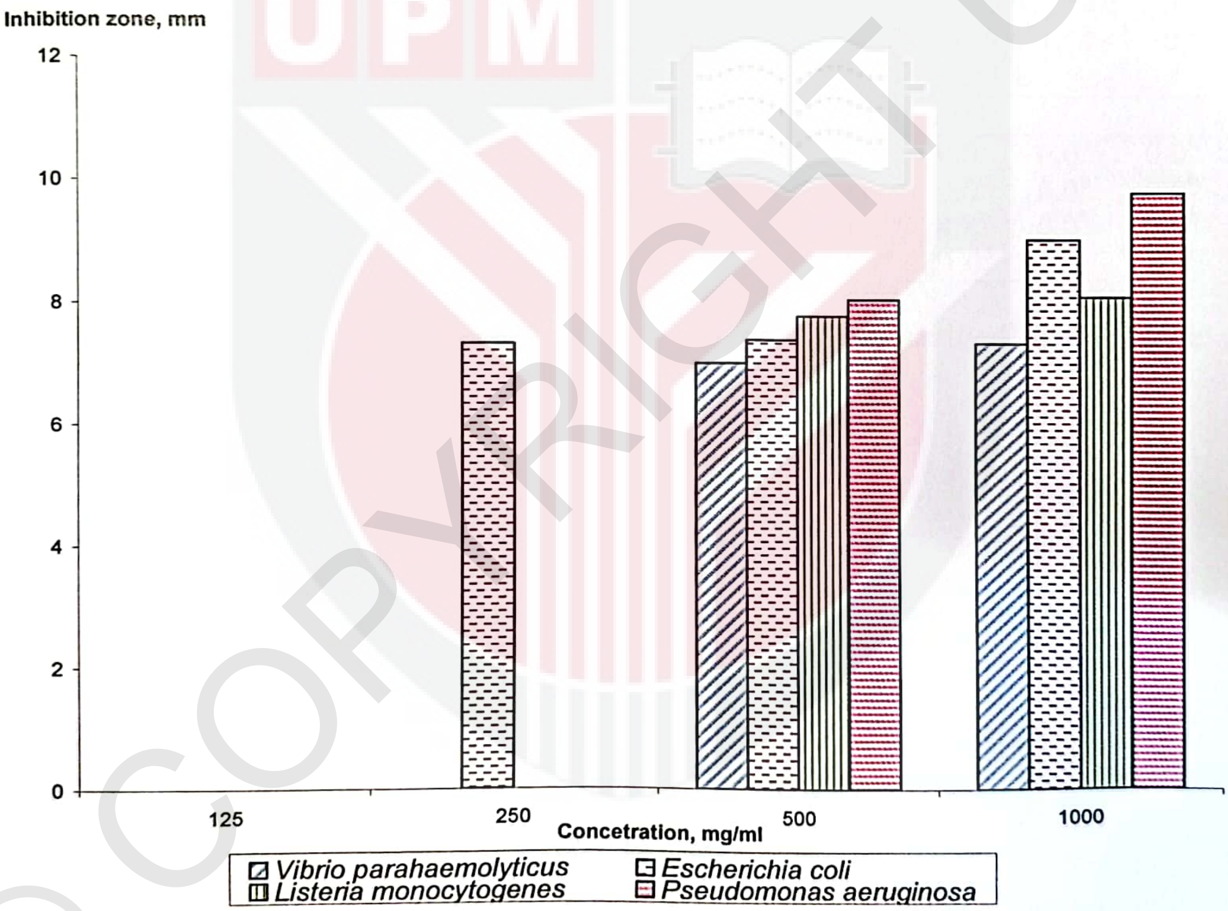

Figure 9: Inhibitory effects *A. excelsa* (leaf) against four bacterial strains

Table 9: Inhibitory effects of crude extracts against the four strains of bacteria

Plant extracts	Concentration, mg/ml								
	1000	500	250	125	62.5	31.3	15.7	7.8	3.9
	Vibrio parahaemolyticus								
A. excelsa	7.3^{c}	7.0^{d}	0.0^{d}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{b}	0.0	0.0
S. alata	13.8^{a}	12.1^{b}	10.6^{b}	8.7^{b}	0.0^{c}	0.0^{c}	0.0^{b}	0.0	0.0
C. nardus	13.5^{a}	13.3^{a}	12.8^{a}	12.0^{a}	11.2^{a}	10.0^{a}	6.9^{a}	0.0	0.0
P. amarus	10.4^{b}	9.5^{c}	8.1^{c}	7.9^{b}	7.1^{b}	7.0^{b}	0.0^{b}	0.0	0.0
	Escherichia coli								
A. excelsa	9.1^{c}	7.4^{c}	7.3^{c}	0.0^{b}	0.0^{c}	0.0^{b}	0.0^{c}	0.0^{b}	0.0^{b}
S. alata	9.0^{d}	7.6^{c}	0.0^{d}	0.0^{b}	0.0^{c}	0.0^{b}	0.0^{c}	0.0^{b}	0.0^{b}
C. nardus	14.2^{b}	13.0^{b}	12.1^{b}	11.4^{a}	11.3^{b}	9.6^{a}	8.6^{b}	0.0^{b}	0.0^{b}
P. amarus	16.0^{a}	15.1^{a}	13.6^{a}	12.4^{a}	12.2^{a}	11.1^{a}	11.3^{a}	10.3^{a}	8.7^{a}
	Listeria monocytogenes								
A. excelsa	8.1^{d}	7.8^{c}	0.0^{d}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{b}	0.0^{b}
S. alata	11.2^{c}	8.6^{c}	8.2^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{b}	0.0^{b}
C. nardus	14.1^{b}	12.9^{a}	12.7^{b}	12.3^{b}	10.3^{b}	10.5^{b}	9.6^{b}	8.4^{a}	0.0^{b}
P. amarus	17.9^{a}	14.7^{a}	14.6^{a}	14.0^{a}	13.8^{a}	12.4^{a}	11.0^{a}	9.8^{a}	9.3^{a}
	Pseudomonas aeruginosa								
A. excelsa	9.8^{c}	8.1^{c}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{c}	0.0^{b}	0.0^{b}
S. alata	10.0^{c}	7.9^{c}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{c}	0.0^{b}	0.0^{b}
C. nardus	13.1^{b}	12.6^{b}	12.4^{a}	11.6^{a}	11.5^{a}	11.1^{a}	8.8^{b}	0.0^{b}	0.0^{b}
P. amarus	16.0^{a}	14.7^{a}	12.9^{a}	12.4^{a}	12.0^{a}	11.7^{a}	11.0^{a}	10.2^{a}	8.4^{a}

Means within columns with the same alphabets are not significantly different where $P<0.05$ (NDMRT)

4.3 Cytotoxicity Assay

4.3.1 Brine shrimp lethality

In the present study the brine shrimp lethality of extracts of six selected plants was determined using the procedure of Othman *et al.*, 2006 with some slight modifications. The degree of lethality was found to be directly proportional to the concentration of the extract. Maximum mortalities took place at a concentration of 1000 µg/ml whereas least mortalities were at 10 µg/ml concentration. The general toxicity of LC_{50} between 500 and 1000 µg/ml was considered weak, moderate when the LC_{50} was between 100 and 500 µg/ml and strong when LC_{50} ranged from 0 to 100 µg/ml (Canales *et al.*, 2006).

The brine shrimp lethality assay showed the methanolic extract leaves of *P. amarus* to be most active among the six plant extracts, killing the shrimp at lower concentration (LC_{50} = 203.1 µg/ml). The stem extracts of *S. alata* and *A. excelsa* were considered moderate toxic with LC_{50} 415.1 µg/ml and LC_{50} 573.4 µg/ml, respectively. While the rest were *C. nardus*, the leaf extracts of *A. excelsa* and *S. alata* were considered weak with LC_{50} 680.8 µg/ml, LC_{50} 1000.0 µg/ml, LC_{50} 1173.0 µg/ml respectively.

Table 10: Brine shrimp lethality data of selected plants

Plants	Part used	Brine shrimp lethality (LC_{50}, µg/ml 24h)
Phyllanthus amarus	Leaf	203.1
Azadirachta excelsa	Leaf	1000.0
Azadirachta excelsa	Stem	573.4
Cymbopogon nardus	Leaf	680.8
Senna alata	Leaf	1173.0
Senna alata	Stem	415.5

4.3.2 Extracts of *Phullanthus amarus*

Table 11: The cytotoxic effects of plant extract of *P. amarus*

	Concentration, µg/ml		
	1000	100	10
Viability %	30	60	83
LC_{50}		203.1	

*LC_{50} determined by Probit Analysis (Appendix 6)

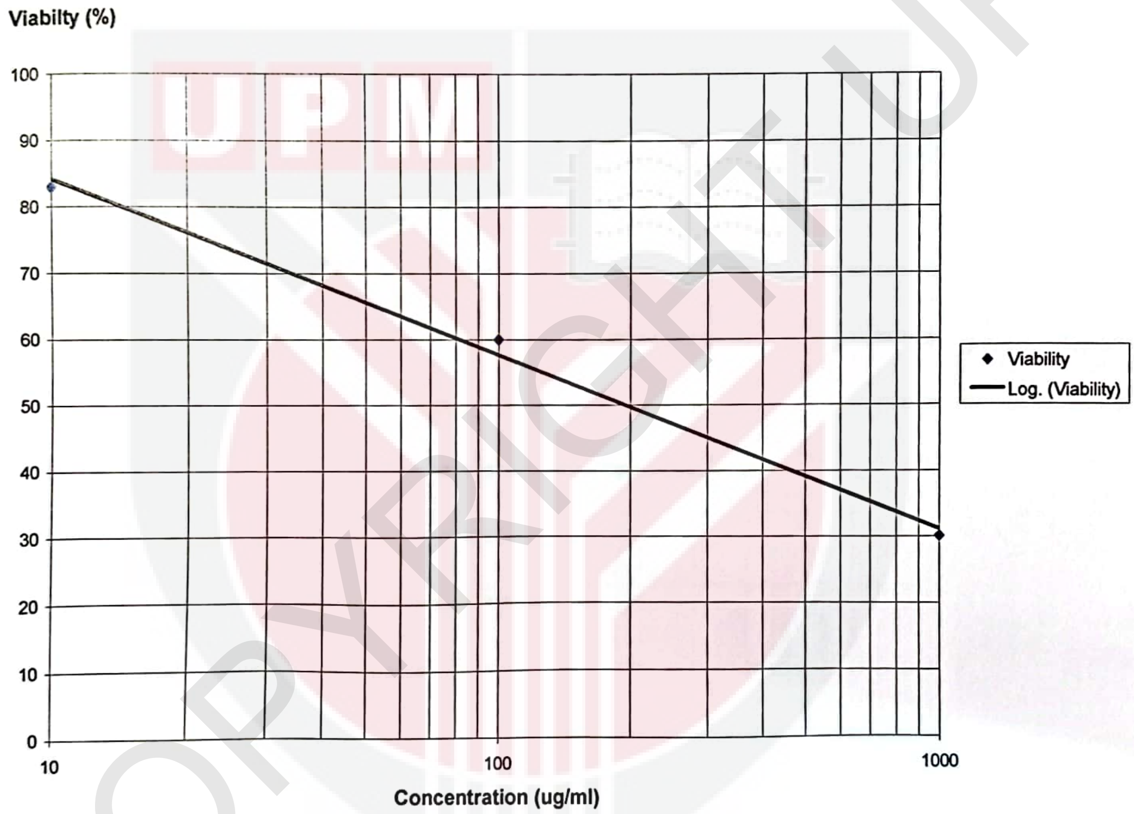

Figure 10: The cytotoxic effects of plant extract of *P. amarus*

4.3.3 Extracts of *Azadirachta excelsa* (leaf)

Table 12: The cytotoxic effects of plant extract of *A. excelsa* (leaf)

	Concentration, µg/ml		
	1000	100	10
Viability %	50	77	77
LC_{50}		**1000.0**	

*LC $_{50}$ determined by using Probit Analysis (Appendix 6)

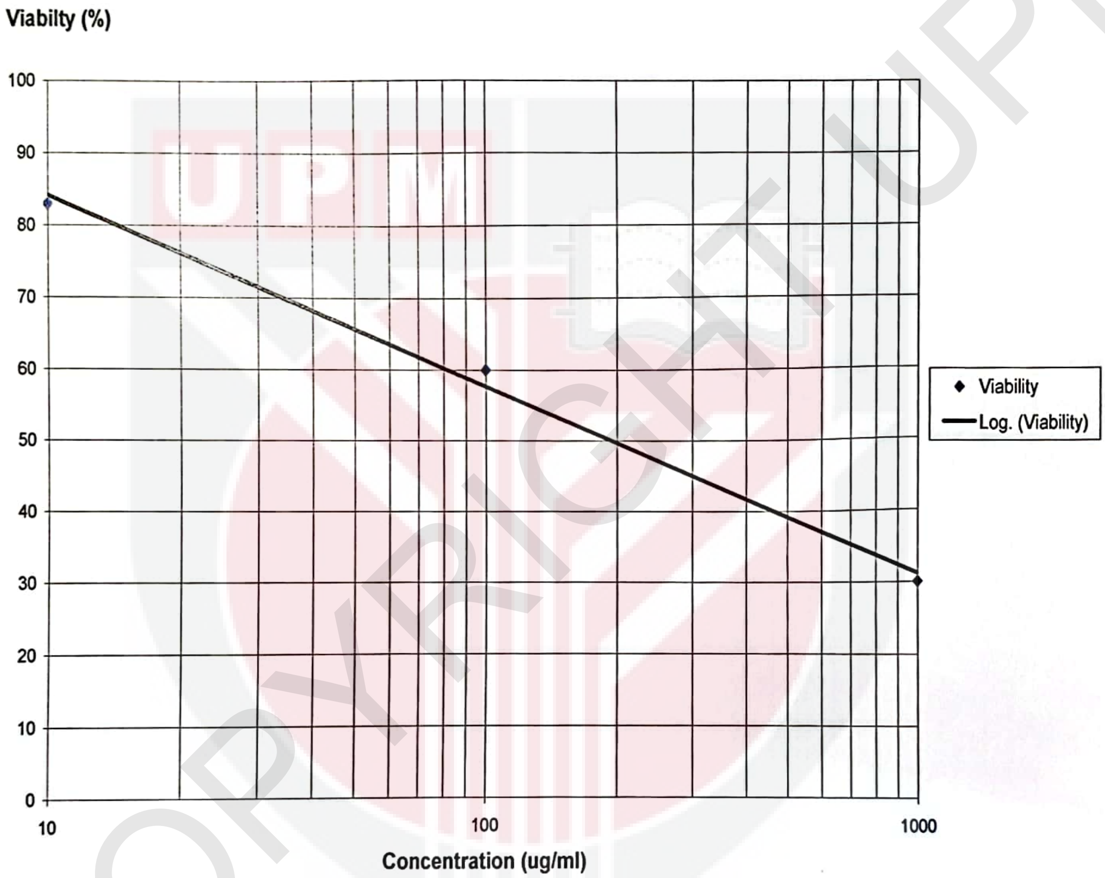

Figure 11: Inhibitory effects of *A. excelsa* (leaf)

4.3.4 Extracts of *Azadirachta excelsa* (stem)

Table 13: The cytotoxic effects of plant extract of *A. excelsa* (stem)

	Concentration, µg/ml		
	1000	100	10
Viability %	43	77	80
LC_{50}		**573.4**	

*LC $_{50}$ determined by using Probit Analysis (Appendix 6)

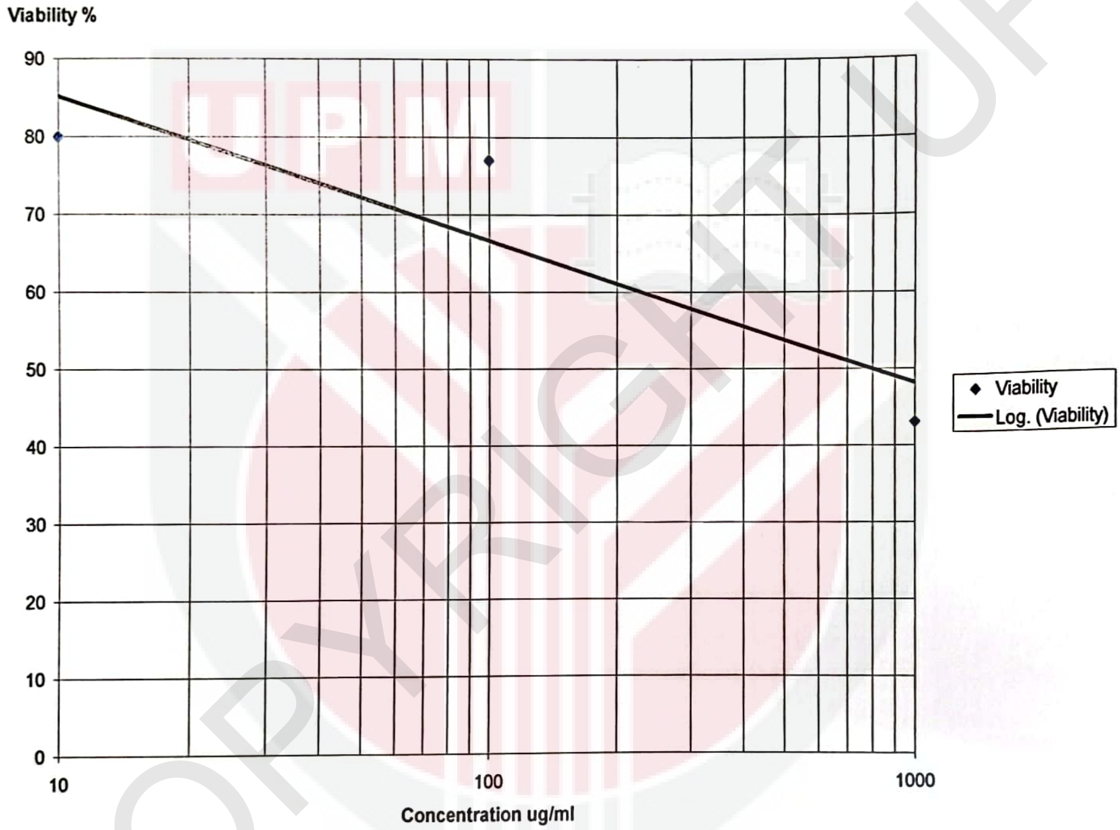

Figure 12: Inhibitory effects of *A. excelsa* (stem)

4.3.5 Extracts of *Cymbopogon nardus*

Table 14: The cytotoxic effects of plant extract of *C. nardus*

	Concentration, µg/ml		
	1000	100	10
Viability %	47	63	73
LC_{50}		**680.8**	

*LC_{50} determined by using Probit Analysis (Appendix 6)

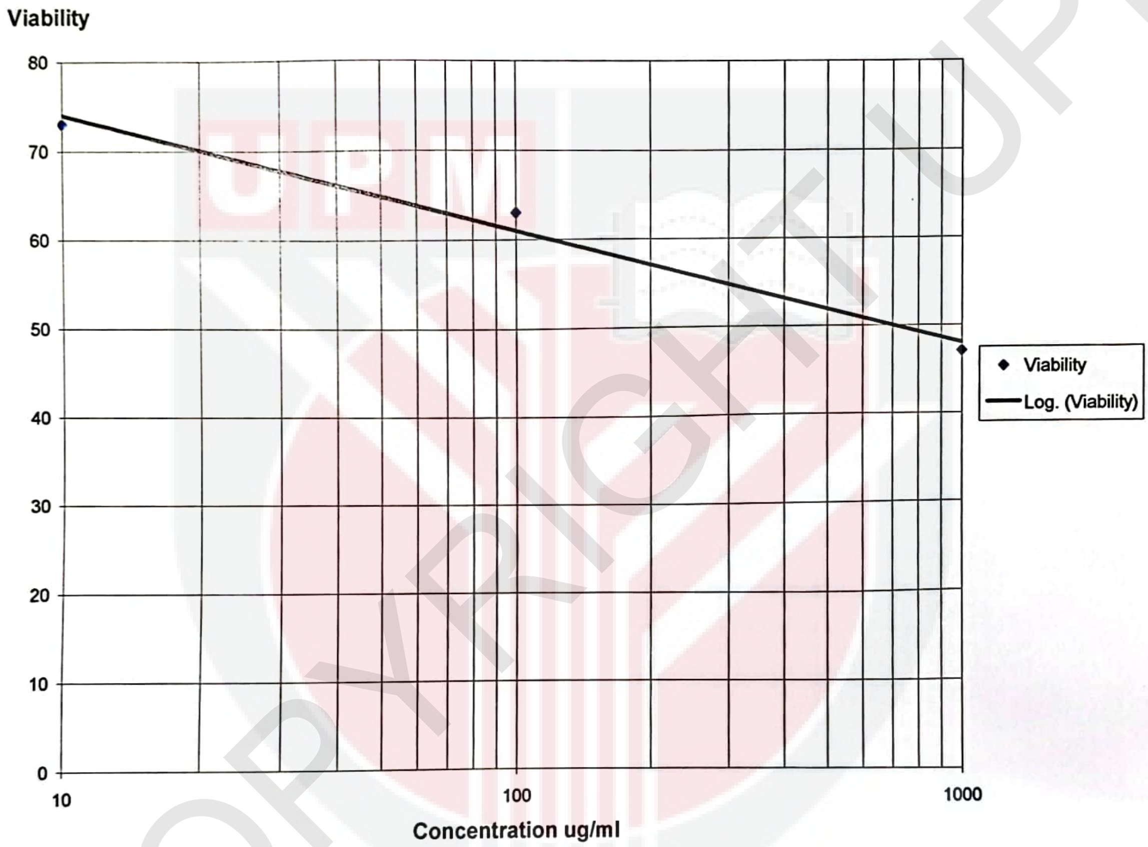

Figure 13: Inhibitory effects of *C. nardus*

4.3.6 Extracts of *Senna alata* (leaf)

Table 15: The cytotoxic effects of plant extract of *S. alata* (leaf)

	Concentration, µg/ml		
	1000	100	10
Viability %	47	63	73
LC_{50}		**1173.0**	

*LC_{50} determined by using Probit Analysis (Appendix 6)

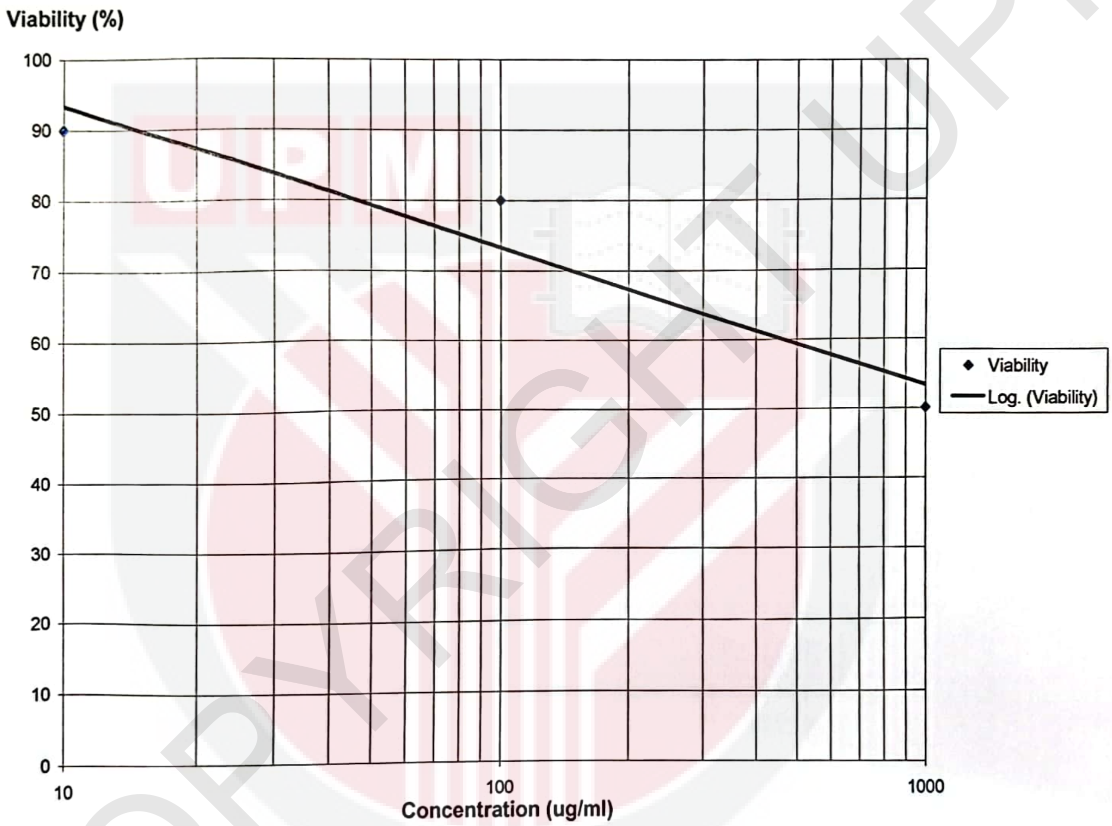

Figure 14: Inhibitory effects of *S. alata* (leaf)

4.3.7 Extracts of *Senna alata* (stem)

Table 16: The cytotoxic effects of plant extract of *S. alata* (stem)

	Concentration, µg/ml		
	1000	100	10
Viability %	47	63	73
LC_{50}		415.5	

*LC 50 determined by using Probit Analysis (Appendix 6)

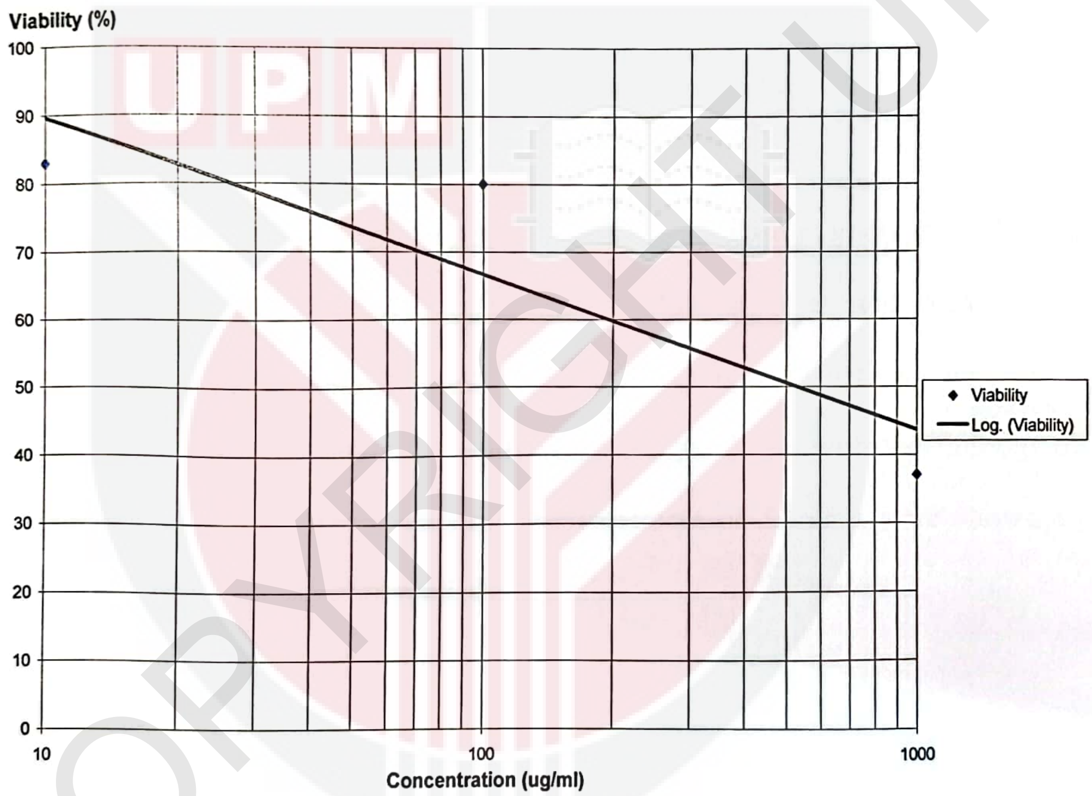

Figure 15: Inhibitory effects of *S. alata* (stem)

CHAPTER 5

DISCUSSION

5.1 Antibacterial Assay

Results of the present study revealed that the methanolic crude extracts of leaves of *P. amarus*, *C. nardus*, *S. alata* and *A. excelsa* possessed potential growth inhibitory activities against *V. parahaemolyticus, E. coli, L. monocytogenes* and *P. aeruginosa*, whereas the stem of *S. alata* was selective against *Vibrio parahaemolyticus*. The stem of *A. excelsa* was inactive against all four bacterial strains.

In this preliminary study on antibacterial activity of six plant extracts, five showed antibacterial properties which confirm their popular use and justify the ethnobotanical approach in the search for novel biologically active compounds. Data of *S. alata* obtained from this study were in accordance with the findings of Owoyale *et al*., (2005), where the crude leaf extracts of *S. alata* were shown to inhibit *E. coli* at the concentration of 125 mg/ml.

Of all the plants studied, the most active antibacterial extract was from *Phyllanthus amarus*. The crude extracts of *P. amarus* inhibited *E. coli, L. monocytogenes* and *P. aeruginosa* at a low concentration of 3.9 mg/ml. The plants evaluated in this study have been reported to possess a number of medicinal properties. *Phyllanthus amarus* is also reported to have antitumour and anticarcinogenic (Rajeshkumar *et al*., 2002), antibacterial properties (Kloucek *et al*., 2005), antinociception (Adair *et al*., 2000) and antifungal properties (Hoffman *et al*., 2004). Kloucek *et al*,. (2005), reported that among compounds isolated from *P. amarus*, tannins, corilagin, geraniin and garlic acid showed in vitro activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*. Many of these are biologically active. From this study, the ability of *P. amarus* to inhibit the growth of all the tested bacteria might be due to one or more of these biologically active compounds that have been previously isolated. However, further phytochemical studies are required to determine the specific of compounds responsible for the antibacterial effects of this species.

Cymbopogon nardus was identified to have potent antibacterial activity and their lowest concentration was 7.8 mg/ml against *L. monocytogenes* and three other bacteria at 15.7 mg/ml. The essential oils present in *C. nardus* are complex mixture comprising of many compounds and each constituent contributes to antibacterial properties. Previous studies using disc diffusion method had revealed that citronella oil exhibited antibacterial and antifungal activities (Nakahara *et al*., 2003). Citronella oil can be a good source of antibacterial agents in addition to its insect repellent properties. Besides that, essential oils are potential sources of novel antimicrobial compounds, especially against bacterial pathogens (Prabuseenivasan *et al*., 2006). From previous literature, this essential oil in *C. nardus* is reported to cause inhibitory activity against *L. monocytogenes* and prevent listeriosis. This gives credence to its enthopharmacological use as a remedy to treat infections and diseases caused by this bacterium.

Results obtained in this study on antibacterial activity of S. *alata*, agree with those obtained by Owoyale *et al*., (2005), who reported that the methanolic leaf extract of *S. alata* showed antibacterial activity against *E. coli* and *P. aeruginosa* at 125 mg/ml but there are no reports on *V. parahaemolyticus* and *Listeria monocytogenes*. But in this present study, the extract was active against all tested bacterial strains. Stem of *S. alata* was selective against *V. parahaemolyticus* at a minimum concentration of 25 mg/ml. This tends to show that the active ingredients in the leaves were more potent than in the stems. According to Ahmad and Osman (1994), leaf extract of *S. alata* was active against fungal and bacterial strains, but yeast strains were not affected by the extracts. The antifungal properties were due to the presence of chrysophanol in the leaf extract. The antifungal properties were the reason why traditional medicine practitioners used *Senna alata* leaf extract to treat ringworm, scabies and other skin diseases. In recent studies (Pieme *et al*., 2006), the safety of utilizing leaf of *S. alata* as medicinal plant to treat various diseases have been proven to be safe.

Among the leaf extracts tested, *A. excelsa* was the least sensitive against the four bacterial strains. *A. excelsa* required a higher concentration or 250 mg/ml to show inhibition against *V. parahaemolyticus* and 500 mg/ml against the rest of the bacterial strains. This suggests that the amount of active constituents present in the leaves may be lower compared to the other species. On the other hand, stem extract of *A. excelsa* had no antibacterial activity, probably due to absence of the active constituents in the stem. Numerous studies have shown *A. excelsa* to have several useful agricultural properties, such as timber species, source of insecticide to protect cabbage and other related *Brassicae* vegetables in Thailand (Kanokmedhakul *et al*., 2005) and as a reforestation species (Morimoto *et al*., 2005). Introduction of modern technology including biotechnology has enabled *In vitro* propagation to produce large numbers of high quality and uniform *A. excelsa* planting stock to the plantation industry (Chin and Othman, 2005).

The results of the present investigations clearly indicate that the antibacterial activity varies with the plant species. It can be concluded that all leaf extracts showed promising antibacterial properties while only the stem extract of *S. alata* showed antibacterial property. The plants tested have potential to generate novel metabolites and may help in the discovery of new chemical classes of antibacterial agents useful to human health and serve as biochemical tools for the study of infectious diseases. The active principles of these plant extracts should be tested for their safety and efficacy to uncover their therapeutic potential in modern and traditional medicine against infectious diseases. The screening of natural products and identifying of active agents is important in producing of lead molecules and drugs.

5.2 Cytotoxicity Assay

Out of the several plants screened for toxicity against the brine shrimp, only one extract *P. amarus* (LC_{50} = 203.1 µg/ml) exhibited LC_{50} < 250.0 µg/ml and this was classified as potent by Pisitthanan *et al*., (2004), and they are considered active and has the potential for further investigation. Many biological effects have been reported for extracts of *P. amarus*, especially cytotoxic effects against human cancer cell lines (Leite *et al.,* 2006) and effects on epithelial transport is also being tested (Sousa *et al*., 2006).

Activity of stem extract *S .alata and A. excelsa* was moderate toxicity with LC_{50} = 415.5 µg/ml, LC_{50} = 573.4 µg/ml, respectively. There are several research have reported in the genus of Azadirachta (Cui *et al*., 97). The bioactive principles that exist in the genus can be expected to be present in the tested plants in this study.

Future investigations should include to structural elucidation of the bioactive compounds. While *C. nardus* (LC_{50} = 680.8 µg/ml), leaf extracts of *A. excelsa* with LC_{50} = 1000.0 µg/ml whereas the least active was the leaf extract of *S. alata* (LC_{50} = 1173.0 µg/ml).

In general, the brine shrimp lethality test is useful for screening plants extracts in order to predict their toxicity before further investigation of the bioactive compounds. This primary bioassay is a prerequisite before further investigation against human cancer cell lines and the leaf and stem extracts lethal to brine shrimps should give positive results on cancer cell lines. The brine shrimp lethality test appears to be a simple test to provide a front-line screen that can be backed up by more specific and expensive bioassays once the bioactive fractions have been identified. The test is effective and useful for quick confirmation of active principles in the extracts. Hopefully, some beneficial product may offer a remedy for the treatment of cancer.

CHAPTER 6

CONCLUSION

Preliminary screening of six plant extracts from four species showed potential antibacterial properties where the most active antibacterial extract was *P. amarus*, while the stem extracts of *A. excelsa* was inactive against the four bacterial strains. *Phyllanthus amarus* was the most active among all plant extracts to inhibit the growth of all the bacteria tested and has strong potential for discovery of antibacterial principles.

In brine shrimp lethality test of the crude extracts, only *P. amarus* exhibited potent activity with LC_{50} values < 250 µg/ml. Based on the possible relationship between brine shrimp and plant bioactivity, this work could serve for further ethnobotanical and phytochemical research. While for all the plants studied, it can be concluded that they have good potential as antibacterial compounds against microorganisms and might be used in the treatment of infectious diseases caused by microorganisms.

The results obtained in this work, showed that the plants studied have potential for development of antibacterial agents rather than anticancer agents. As the extracts exhibited potent activity against bacteria compared to cytotoxicity effects on brine shrimp.

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APPENDICES A

Appendix 1: Antibacterial activities of methanolic extracts of *Phyllanthus amarus*

Concentration, mg/ml	Replication	Inhibition zone diameter (mm) produced by plant extracts											
		Vibrio parahaemolyticus			*Escherichia coli*			*Listeria monocytogenes*			*Pseudomonas auruginosa*		
1000	1	12.0	11.0	11.0	18.0	18.0	15.0	22.0	15.0	19.0	14.0	17.0	16.0
	2	10.0	10.0	10.0	17.0	14.0	14.0	19.0	20.0	17.0	13.0	17.0	17.0
	3	10.0	10.0	10.0	18.0	16.0	14.0	17.0	18.0	14.0	14.0	20.0	16.0
	Mean	**10.4**			**16.0**			**17.9**			**16.0**		
	SD	0.7			1.7			2.3			2.0		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		
500	1	11.0	9.0	9.0	12.0	16.0	18.0	13.0	15.0	16.0	13.0	15.0	16.0
	2	9.0	9.0	10.0	17.0	13.0	17.0	13.0	16.0	15.0	13.0	16.0	15.0
	3	9.0	9.0	11.0	14.0	13.0	16.0	15.0	15.0	14.0	15.0	15.0	14.0
	Mean	**9.5**			**15.1**			**14.7**			**14.7**		
	SD	0.8			2.0			1.4			1.1		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		
250	1	8.0	8.0	9.0	12.0	13.0	18.0	14.0	13.0	15.0	11.0	15.0	15.0
	2	8.5	8.0	8.0	12.0	13.0	16.0	14.0	14.0	18.0	12.0	15.0	14.0
	3	8.0	8.0	7.0	10.0	13.0	15.0	13.0	15.0	15.0	11.0	11.0	12.0
	Mean	**8.1**			**13.6**			**14.6**			**12.9**		
	SD	0.5			2.3			1.4			1.7		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		
125	1	7.5	8.0.	7.0	10.0	13.0	14.0	15.0	13.0	14.0	11.0	13.0	15.0
	2	7.0	7.0	8.0	12.0	10.0	15.0	13.0	14.0	15.0	11.0	12.0	14.0
	3	8.0	7.0	9.0	11.0	12.0	15.0	14.0	13.0	15.0	11.0	13.0	12.0
	Mean	**7.9**			**12.4**			**14.0**			**12.4**		
	SD	0.7			1.8			0.8			1.3		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		

Concentration, mg/ml	Replication	Inhibition zone diameter (mm) produced by plant extracts											
		Vibrio parahaemolyticus			*Escherichia coli*			*Listeria monocytogenes*			*Pseudomonas auruginosa*		
62.5	1	7.0	7.0	7.0	12.0	12.0	13.0	13.0	13.0	14.0	11.0	13.0	13.0
	2	7.0	7.0	7.0	12.0	13.0	12.0	15.0	14.0	13.0	12.0	11.0	10.0
	3	8.0	7.0	7.0	12.0	13.0	11.0	15.0	13.0	14.0	11.0	14.0	13.0
	Mean	**7.1**			**12.2**			**13.8**			**12.0**		
	SD	0.3			0.6			0.8			1.3		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		
31.3	1	7.0	7.0	7.0	8.0	12.0	14.0	14.0	14.0	12.0	9.0	13.0	13.0
	2	7.0	7.0	7.0	8.0	12.0	12.0	12.0	11.0	13.0	11.0	14.0	11.0
	3	7.0	7.0	7.0	8.0	11.0	15.0	12.0	12.0	12.0	9.0	13.0	12.0
	Mean	**7.0**			**11.1**			**12.4**			**11.7**		
	SD	0.0			2.5			1.0			1.7		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		
15.7	1	0.0	0.0	0.0	12.0	11.0	12.0	12.0	9.0	13.0	13.0	10.0	13.0
	2	0.0	0.0	0.0	11.0	11.0	11.0	11.0	11.0	12.0	11.0	11.0	11.0
	3	0.0	0.0	0.0	12.0	12.0	10.0	10.0	10.0	11.0	11.0	10.0	14.0
	Mean	**0.0**			**11.3**			**11.0**			**11.6**		
	SD	0.0			0.7			1.8			1.3		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		
7.8	1	0.0	0.0	0.0	11.0	10.0	10.0	10.0	8.0	10.0	10.0	9.0	11.0
	2	0.0	0.0	0.0	11.0	11.0	9.0	12.0	9.0	11.0	10.0	10.0	11.0
	3	0.0	0.0	0.0	10.0	11.0	10.0	9.0	9.0	10.0	10.0	10.0	11.0
	Mean	**0.0**			**10.3**			**9.8**			**10.2**		
	SD	0.0			0.6			1.1			0.6		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		

Concentration, mg/ml	Replication	Inhibition zone diameter (mm) produced by plant extracts											
		Vibrio parahaemolyticus			*Escherichia coli*			*Listeria monocytogenes*			*Pseudomonas auruginosa*		
3.9	1	0.0	0.0	0.0	8.0	10.0	8.0	11.0	8.0	8.0	8.0	9.0	8.0
	2	0.0	0.0	0.0	9.0	9.0	9.0	9.0	11.0	9.0	10.0	8.0	8.0
	3	0.0	0.0	0.0	8.0	9.0	8.0	9.0	9.0	10.0	9.0	7.0	9.0
	Mean	**0.0**			**8.7**			**9.3**			**8.4**		
	SD	0.0			0.9			1.1			0.8		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		
1.9	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	**0.0**			**0.0**			**0.0**			**0.0**		
	SD	0.0			0.0			0.0			0.0		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		

Appendix 2: Antibacterial activity methanolic extracts of *Cymbopogon nardus*

Concentration, mg/ml	Replication	Inhibition zone diameter (mm) produced by plant extracts											
		Vibrio parahaemolyticus			*Escherichia coli*			*Listeria monocytogenes*			*Pseudomonas auruginosa*		
1000	1	11.0	14.0	14.0	15.0	16.0	15.0	14.0	19.0	12.0	11.0	15.0	12.0
	2	13.0	13.0	15.0	13.0	14.0	16.0	11.0	15.0	13.0	14.0	12.0	15.0
	3	14.0	13.0	14.5	13.0	11.0	15.0	15.0	12.5	15.0	12.0	13.0	14.0
	Mean	**13.5**			**14.2**			**14.1**			**13.1**		
	SD	1.1			1.6			2.2			1.4		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		
500	1	10.0	13.0	15.0	12.0	15.0	11.0	13.0	18.0	11.0	11.0	13.0	14.0
	2	13.0	13.0	14.0	14.0	13.0	14.0	10.0	13.0	12.5	11.0	12.0	12.0
	3	13.0	14.0	15.0	13.0	13.0	12.0	13.0	12.0	14.0	13.0	12.0	15.0
	Mean	**13.3**			**13.0**			**12.9**			**12.6**		
	SD	1.4			1.2			2.1			1.3		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		
250	1	11.0	11.0	15.0	11.0	11.0	11.0	14.0	17.0	15.0	11.0	13.0	14.0
	2	13.0	13.0	14.0	12.0	13.0	14.0	10.0	12.0	10.0	11.0	11.0	14.0
	3	12.0	13.0	13.0	10.0	13.0	14.0	13.0	11.0	12.0	12.0	11.0	15.0
	Mean	**12.8**			**12.1**			**12.7**			**12.4**		
	SD	1.3			1.4			2.2			1.5		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		
125	1	10.0	11.0	14.0	12.0	10.0	11.0	12.0	12.0	13.0	10.0	11.0	13.0
	2	12.0	12.0	14.0	11.0	14.0	13.0	11.0	14.0	11.0	10.0	11.0	12.0
	3	11.0	11.0	13.0	10.0	10.0	12.0	13.0	12.0	13.0	10.0	11.0	16.0
	Mean	**12.0**			**11.4**			**12.3**			**11.6**		
	SD	1.3			1.3			0.9			1.8		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		

Concentration, mg/ml	Replication	Inhibition zone diameter (mm) produced by plant extracts											
		Vibrio parahaemolyticus			*Escherichia coli*			*Listeria monocytogenes*			*Pseudomonas auruginosa*		
62.5	1	12.0	13.0	11.0	9.0	9.0	11.0	9.0	12.0	15.0	11.0	12.0	11.0
	2	11.0	11.0	11.0	9.0	9.0	10.0	9.0	12.0	14.0	12.0	11.0	11.0
	3	11.0	11.0	10.0	9.0	9.0	11.0	9.0	11.0	17.0	11.0	12.0	12.0
	Mean	**11.2**			**11.3**			**10.3**			**11.5**		
	SD	0.8			1.0			2.8			0.5		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		
31.3	1	10.0	11.0	9.0	8.0	10.0	12.0	8.0	11.0	13.0	10.5	12.0	11.0
	2	10.0	11.0	9.0	8.5	12.0	13.0	9.0	10.5	11.0	11.0	11.0	11.0
	3	9.0	11.0	10.0	9.0	11.0	10.0	8.0	10.0	14.0	9.0	12.0	12.0
	Mean	**10.0**			**9.6**			**10.5**			**11.1**		
	SD	0.8			1.7			1.9			0.9		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		
15.7	1	10.0	11.0	9.0	9.0	8.0	9.0	11.0	9.0	9.0	8.0	8.0	7.0
	2	10.0	11.0	9.0	8.0	7.5	8.0	10.0	9.0	9.0	10.0	9.0	10.0
	3	9.0	11.0	10.0	10.0	9.0	9.0	10.0	10.0	9.0	9.0	9.0	9.0
	Mean	**6.9**			**8.6**			**9.6**			**8.8**		
	SD	0.9			0.7			0.7			0.9		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		
7.8	1	0.0	0.0	0.0	0.0	0.0	0.0	11.0	9.0	9.0	0.0	0.0	0.0
	2	0.0	0.0	0.0	0.0	0.0	0.0	10.0	9.0	9.0	0.0	0.0	0.0
	3	0.0	0.0	0.0	0.0	0.0	0.0	10.0	10.0	9.0	0.0	0.0	0.0
	Mean	**0.0**			**0.0**			**8.4**			**0.0**		
	SD	0.0			0.0			1.0			0.0		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		

Appendix 3: Antibacterial activity of methanolic extracts of *Senna alata* (leaf)

Concentration, mg/ml	Replication	Inhibition zone diameter (mm) produced by plant extracts											
		Vibrio parahaemolyticus			*Escherichia coli*			*Listeria monocytogenes*			*Pseudomonas auruginosa*		
1000	1	14.0	14.0	14.0	8.0	9.0	9.0	11.0	12.0	10.0	10.0	10.0	10.0
	2	14.0	14.0	14.0	8.0	9.5	10.0	11.0	11.0	12.0	10.0	9.0	12.0
	3	13.0	13.0	14.0	7.0	9.0	9.0	11.0	11.0	12.0	9.0	10.0	10.0
	Mean	**13.8**			**9.0**			**11.2**			**10.0**		
	SD	0.4			1.4			0.6			0.8		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		
500	1	12.5	12.5	13.0	8.5	7.0	8.0	9.0	8.0	9.0	7.5	8.0	9.0
	2	12.0	12.0	13.0	8.0	6.0	7.0	8.0	7.0	9.0	7.0	8.0	9.0
	3	11.0	12.0	11.0	8.0	8.0	8.0	8.0	9.0	10.0	7.0	8.0	8.0
	Mean	**12.1**			**7.6**			**8.6**			**7.9**		
	SD	0.7			0.7			0.8			0.7		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		
250	1	11.0	11.0	11.0	0.0	0.0	0.0	11.0	8.0	7.0	0.0	0.0	0.0
	2	9.5	11.0	11.0	0.0	0.0	0.0	8.0	7.0	8.0	0.0	0.0	0.0
	3	10.0	10.0	11.0	0.0	0.0	0.0	9.0	8.0	8.0	0.0	0.0	0.0
	Mean	**10.6**			**0.0**			**8.2**			**0.0**		
	SD	0.6			0.0			1.1			0.0		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		
125	1	8.0	9.0	9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2	8.0	9.5	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3	7.0	9.0	9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	**8.7**			**0.0**			**0.0**			**0.0**		
	SD	0.9			0.0			0.0			0.0		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		

Concentration, mg/ml	Replication	Inhibition zone diameter (mm) produced by plant extracts											
		Vibrio parahaemolyticus			*Escherichia coli*			*Listeria monocytogenes*			*Pseudomonas auruginosa*		
62.5	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	**0.0**			**0.0**			**0.0**			**0.0**		
	SD	0.0			0.0			0.0			0.0		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		

Appendix 4: Antibacterial activity of methanolic extracts of *Senna alata* (stem)

Concentration, mg/ml	Replication	Inhibition zone diameter (mm) produced by plant extracts											
		Vibrio parahaemolyticus			*Escherichia coli*			*Listeria monocytogenes*			*Pseudomonas auruginosa*		
100	1	12.0	11.0	11.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2	12.0	12.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3	10.0	11.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	**11.0**			**0.0**			**0.0**			**0.0**		
	SD	0.8			0.0			0.0			0.0		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		
50	1	9.0	8.0	9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2	9.0	8.0	9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3	8.5	8.0	9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	**8.6**			**0.0**			**0.0**			**0.0**		
	SD	0.5			0.0			0.0			0.0		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		
25	1	8.0	8.0	8.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2	7.5	7.5	7.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3	7.0	7.0	8.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	**7.6**			**0.0**			**0.0**			**0.0**		
	SD	0.4			0.0			0.0			0.0		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		
12.5	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	**0.0**			**0.0**			**0.0**			**0.0**		
	SD	0.0			0.0			0.0			0.0		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		

Appendix 5: Antibacterial activity of methanolic extracts of *Azadirachta excelsa* (leaf)

Concentration, mg/ml	Replication	Inhibition zone diameter (mm) produced by plant extracts											
		Vibrio parahaemolyticus			*Escherichia coli*			*Listeria monocytogenes*			*Pseudomonas auruginosa*		
1000	1	8.0	6.5	7.0	10.0	8.0	10.0	9.0	8.0	7.0	13.0	8.5	9.0
	2	8.0	8.0	7.0	9.0	9.0	8.0	9.0	8.0	8.0	12.0	8.0	8.0
	3	7.0	8.0	6.5	9.0	8.5	10.0	8.0	8.0	8.0	13.0	9.0	8.0
	Mean	**7.3**			**9.1**			**8.1**			**9.8**		
	SD	0.7			0.8			0.6			2.2		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		
500	1	8.0	6.5	6.5	7.0	8.0	7.0	8.0	8.0	7.0	8.0	8.0	8.0
	2	8.0	7.0	6.5	8.0	6.5	6.5	8.0	7.0	8.0	8.0	8.0	8.0
	3	7.0	7.0	6.5	8.0	8.0	7.5	7.0	9.0	8.0	8.0	8.5	8.0
	Mean	**7.0**			**7.4**			**7.8**			**8.1**		
	SD	0.6			0.7			0.7			0.2		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		
250	1	0.0	0.0	0.0	7.0	7.0	8.0	0.0	0.0	0.0	0.0	0.0	0.0
	2	0.0	0.0	0.0	7.0	8.0	7.0	0.0	0.0	0.0	0.0	0.0	0.0
	3	0.0	0.0	0.0	7.0	7.0	8.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	**0.0**			**7.3**			**0.0**			**0.0**		
	SD	0.0			0.5			0.0			0.0		
	+ Control	+			+			+			+		
	- Control	-			-			-			-		
125	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	**0.0**			**0.0**			**0.0**			**0.0**		
	SD	0.0			0.0			0.0			0.0		
	+ Control	+			+			+			+		

APPENDIX B

Brine Shrimp Lethality Test

Appendix 6: Cytotoxic activity of methanolic plant extracts

Concentration ,µg/ml	1000					100					10				
	1	**2**	**3**	**MEAN (%)**	**Viability (%)**	**1**	**2**	**3**	**MEAN (%)**	**Viability (%)**	**1**	**2**	**3**	**MEAN (%)**	**Viability (%)**
A. excelsa,leaf	50	50	50	50	50	30	0	40	23	77	20	20	30	23	77
A. excelsa,stem	70	60	40	57	43	30	30	10	23	77	30	10	20	20	80
S. alata,leaf	50	50	50	50	50	30	10	20	20	80	10	20	0	10	90
S.alata, stem	60	80	50	63	37	10	30	20	20	80	0	40	10	17	83
P. amarus	80	80	50	70	30	50	40	30	40	60	30	10	10	17	83
C. nardus	60	50	50	53	47	40	30	40	37	63	10	30	40	27	73

Appendix 7: Determination of LC_{50} by Probit Analysis

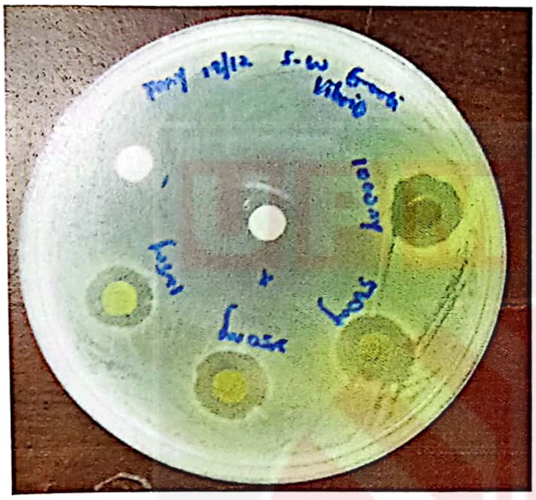
Plant extracts	3	2	1		LC_{50}
A. excelsa,leaf	5.000	4.261	4.261	3.000	1000.0
A. excelsa,stem	4.824	5.739	5.842	2.758	573.4
S. alata,leaf	5.000	4.158	3.718	3.069	1173.0
S.alata, stem	5.332	4.158	4.046	2.619	415.5
P. amarus	5.524	4.747	4.046	2.308	203.1
C. nardus	5.075	4.668	4.387	2.833	680.8

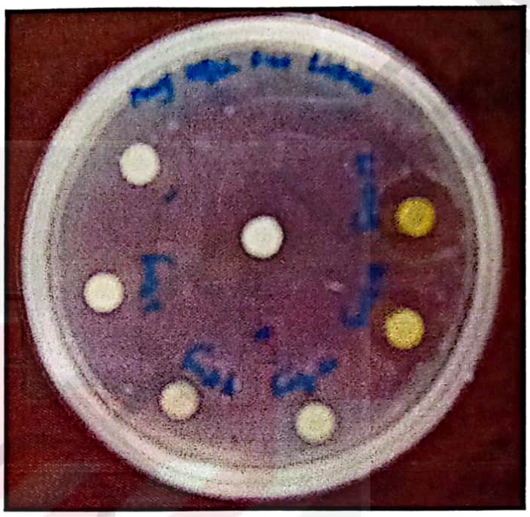
Appendix C

Antibacterial Assay

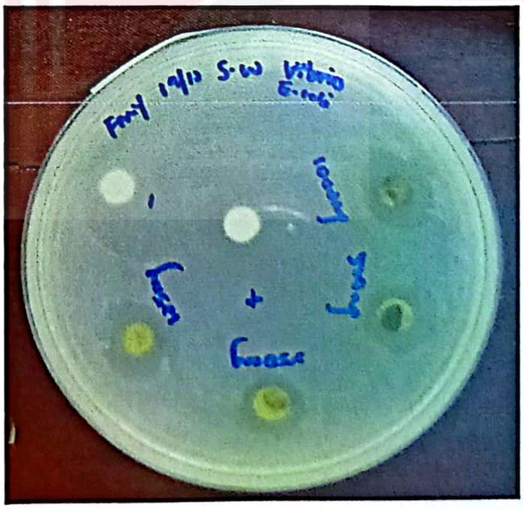
Extracts of *Cymbopogon nardus*

Appendix 8: Inhibitory effects against *Vibrio parahaemolyticus* and *Listeria monocytogenes*



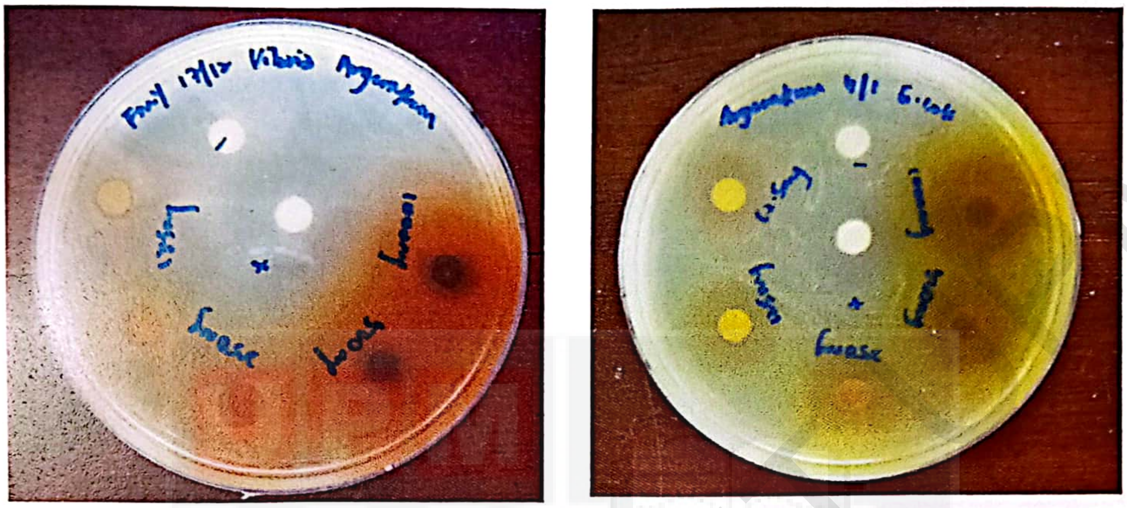


Appendix 9: Inhibitory effects against *Pseudomonas aeruginosa* and *Escherichia coli*

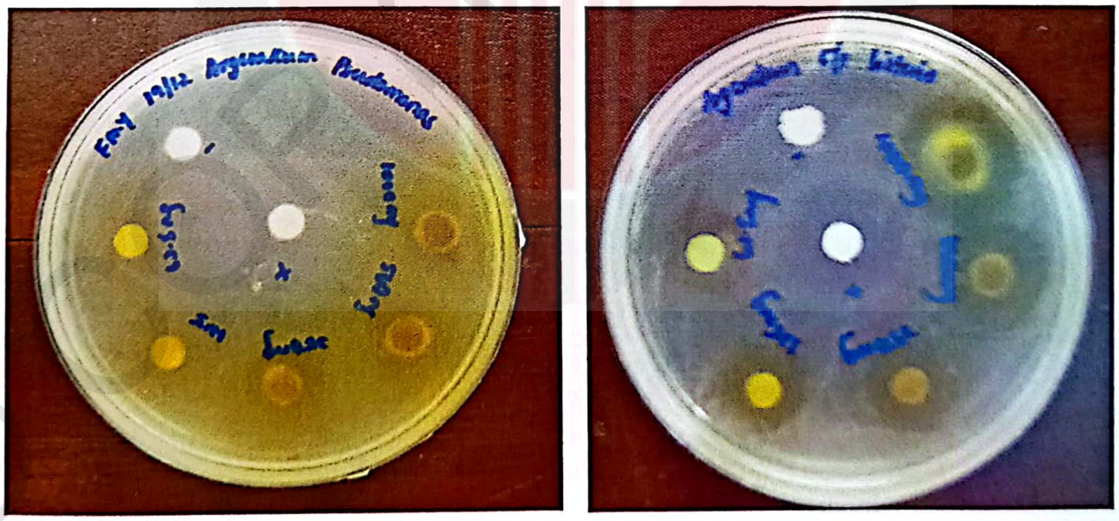


Extracts of *Phyllanthus amarus*

Appendix 10: Inhibitory effects against *Vibrio parahaemolyticus* and *Escherichia coli*

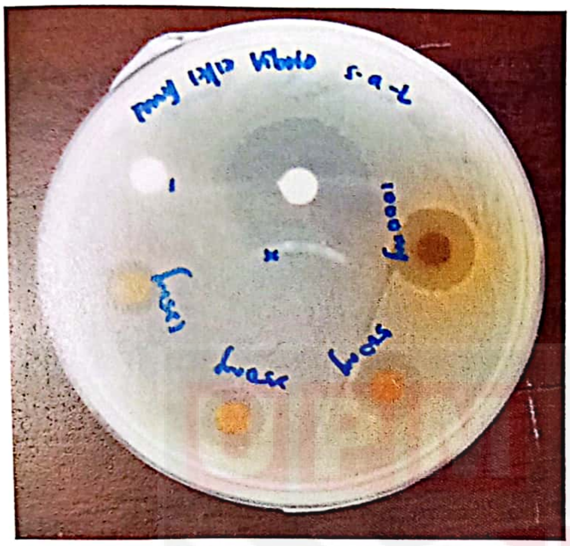


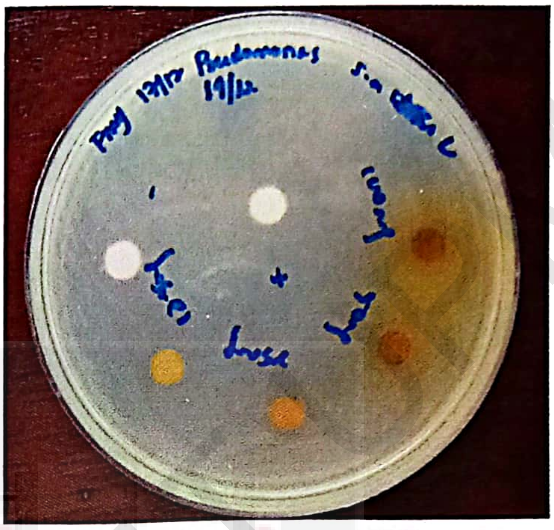
Appendiix 11: Inhibitory effects against *Pseudomonas aeruginosa* and *Listeria monocytogenes*



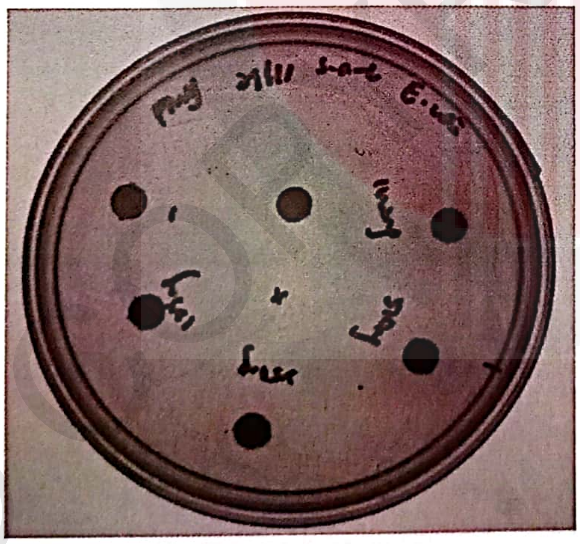
Extracts of *Senna alata* (leaf)

Appendix 12 : Inhibitory effects against *Vibrio parahaemolyticus* and *Pseudomonas aeruginosa*



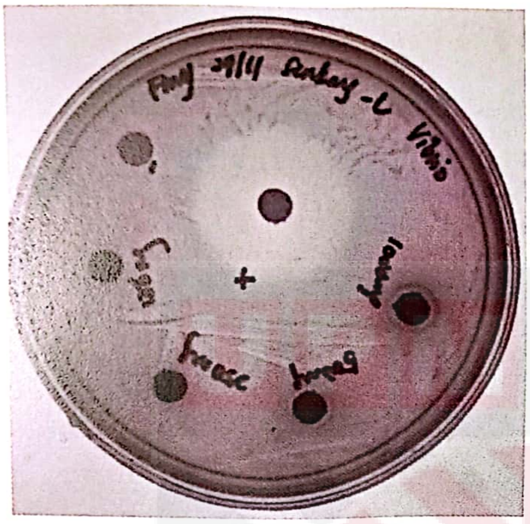


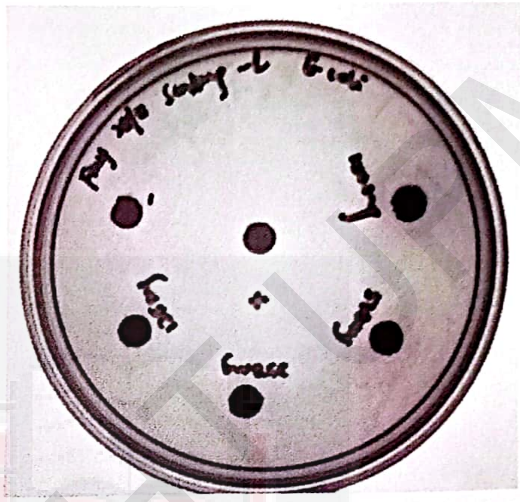
Appendix 13: Inhibitory effects against *Escherichia coli*



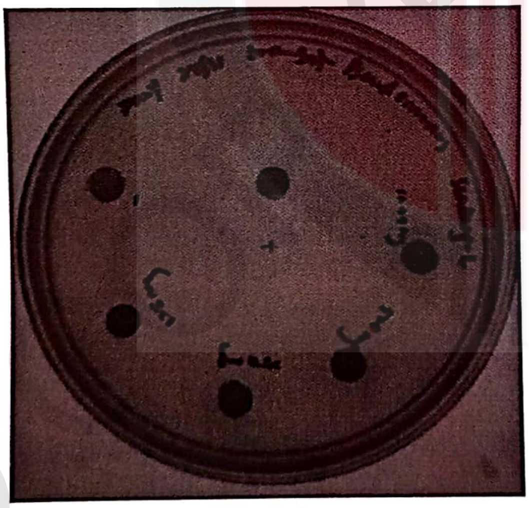
Extract of *Azadirachta excelsa* (leaf)

Appendix 14: Inhibitory effects against *Vibrio parahaemolyticus* and *Escherichia coli*





Appendix 15: Inhibitory effects against *Pseudomonas aeruginosa*



PUBLICATION OF THE PROJECT UNDERTAKING

This is certify that I have no objection to publish the project entitled "Antibacterial and Cytotoxic Activities from Some Selected Plants" by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus and published in the form approved by the faculty.

Foo May Yin

Date: 7 May 2007