COMPARATIVE ANIMICROBIAL EFFECT OF THE POLYHERBAL FORMULATION MACEDAR AND METHANOL EXTRACT OF *Carica papaya* LEAVES

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A PROJECT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES

IN PARTIAL FULFULLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF A BACHELOR OF SCIENCE (B.Sc. HONOURS) SCIENCE LABORATORY TECHNOLOGY.

OCTOBER, 2025

CERTIFICATION

This is to certify that this research work was carried out by **Agbor Joy Abigail** with matriculation number LSC2009868 of the Department of Science Laboratory Technology, Faculty of Life Sciences, and University of Benin, Benin City.

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DEDICATION

I dedicate this work to Almighty God, who has blessed and sustained me throughout this project work and has been my source of inspiration and strength.

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I sincerely appreciate the guidance and support of my supervisor, Dr. P. O. Obaro and his wife Dr. (Mrs.) O. E. Obaro-Onezeyi, whose valuable insights, encouragement, and constructive criticism played a significant role in shaping this project.

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Abstract

Polyherbal formulations have long been utilized in traditional medicine for their synergistic therapeutic benefits. This study evaluated the comparative antimicrobial activities of Macedar a polyherbal formulation composed of *Carica papaya* leaves, *Veronica amygdalina* leaves, and *Croton hirtus* leaves and *Carica papaya* leaves alone. Methanol extracts were prepared using cold maceration and tested against selected Gram-positive (Staphylococcus aureus, Bacillus cereus) and Gram-negative (Escherichia coli, Pseudomonas aeruginosa) bacteria through agar well diffusion at varying concentrations (62.5–500 mg/ml). The results revealed that both extracts exhibited significant, dose-dependent antibacterial activity, with Macedar demonstrating stronger inhibitory effects across all test organisms. The highest inhibition zones were observed at 500 mg/ml, with Staphylococcus aureus showing the greatest susceptibility. These findings indicate that the synergistic interaction of the three plants in Macedar enhances antimicrobial potency beyond that of single-plant extracts. The study provides scientific validation for the ethno medicinal use of these plants and highlights the potential of polyherbal formulations Macedar as effective alternatives in combating resistant bacterial infections.

CHAPTER ONE

1.0 BACKGROUND OF STUDY

The medicinal uses of poly herbal formulations were idealized by Ayurvedic medicine. Poly herbal formulation involves the use of three or more plant mixtures with various herbal products to eradicate diseases and their causes, restore balance and create a healthy lifestyle that is helpful in the prevention of recurrence of imbalance (Parasuraman *et al.,* 2014).

Macedar was idealized from by the researcher from the basic knowledge of antimicrobial activities of the single plants from the polyherbal formulation of Macedar which is a combination of *Carica papaya* tree bark, *Vernonia amygdalina* Del leaf and *Croton hirtus* leaf.

Phytochemical screening is one of the necessary steps to find out the chemical constituents which lead the isolation of bioactive compounds. Since the 19^{th} century different bioactive constituents of plants have been isolated and characterized to show the medicinal activity as well as physiological activity (Ganguly *et al.,* 2023). These phytochemicals hold the key to the alternative control of even resistant species of bacteria and human pathogens and their uses have been shown to have a scientific basis. Many of these are used as the active ingredients of the modern medicine or as the lead compound for new drug discovery (Mathias *et al.,* 2024).

Bacterial and fungal infections are a serious problem in the world today. Micro-organism related diseases, as at 2016 were ranked amongst the top 10 causes of death in man (WHO, 2018).

Third world countries with inadequate sanitation and primary health care programs are worse hit by microbial infections (Anwana *et al.,* 2022) As a consequence, there has been a sharp increase in the research of anti-microbial effects of different plants which can serve as cure to micro-organism related illness (Anyanwu and Okoye, 2021). Already, at least 40% of pharmaceuticals are gotten from plants, animals, bacteria and fungi (Anwana *et al.,* 2022).

In Nigeria, a lot of plants have been studied for their anti-microbial properties using various methods to carry out this experiment. Examples of such plants include: *Carica papaya* tree bark, *Vernonia amygdalina* Del leaf and *Croton hirtus* leaf *Mallotus oppositifolius, Pterocarpus osun, Vitex doniana, Eucalyptus camaldulensis,Cassia alata, Trichophyton rubrum, Gossypium arboretum,* etc. (Iwu *et. al.,* 2023). It is important to note that in the above named plants study, different parts of the plants were used for the study including, seeds, leaves, stems, barks and roots.

In this study, the Synergistic antimicrobial assessment methanol extracts of the polyherbal formulation of *Carica papaya* tree bark, *Vernonia amygdalina* Del leaf and *Croton hirtus* leaf using Agar Well Diffusion (AWD) method at different concentrations.

1.1 AIM AND OBJECTIVES

1.2 AIM

To study the comparative antimicrobial assessment of the aqueous and methanol extracts of *Pentaclethra macrophylla* bark on selected microorganisms

1.2.1 OBJECTIVES

The aim was achieved by the following objectives:

- Carry out the extraction methanol extracts of Polyherbal formulation of Macedar (*Carica papaya* tree bark, *Vernonia amygdalina* Del leaf and *Croton hirtus* leaf) in the ratio of 1:1:1
- Determine thesynergistic antibacterial effect using methanol extracts of Macedar.
- Determine the Minimum Inhibitory Concentration (MIC) and zone of inhibition of the Polyherbal formulation .

CHAPTER TWO

2.0 LITERATURE REVIEW

The use of Plants for the treatment of diseases has been widely done in different parts of the world.Certain plants contain therapeutic properties and traditional medicine utilizes these plants in the treatments of ailments and procurement of cures for diseases. The medicinal properties of plants are as a result of the various array of bioactive compounds, such as alkaloids, flavonoids, tannins, phenolics, and terpenoids present in them.These compounds are sought for their antimicrobial, anti-inflammatory, antioxidant, and anticancer effects (Atanasov et al., 2022). These compounds have been used in both traditional medicine and modern pharmaceutical developments to treat and prevent diseases.

The use of plants for medicinal purposes dates back thousands of years, with evidence from ancient civilizations such as the Egyptians, Chinese, and Indians who documented the use of herbs in treating ailments. An example is, Artemisia annua, used in traditional Chinese medicine, led to the development of artemisinin, a highly effective antimalarial drug (Voorhoeve, 2021). The ongoing use and reference to plants as a source of healing continues to inspire modern drug discovery.

2.1 Description of Vernonia amygdalina Del

Vernonia amygdalina is a wooded shrub of about 2 to 10 m height that regenerates rapidly after planted. The leaves are petiolated in shape with a bitter taste of which its common name “Bitter leaf” springs up (Agbogidi and Akpomorine, 2019).

Bitter leaf scientifically known as *Vernonia amygdalina* is one of the most famous plants found in Africa and Asia. It is the most cultivated species of the genus Vernonia that is about 1,000 species of shrub (Agbogidi and Akpomorine, 2019; Toyang and Verpoorte, 2013; Egharevba *et al.,* 2014 and Njan *et al.,* 2018). *Vernonia amygdalin*a has been the most prominent species in the family of Asteraceae that had been studied in Africa (Ankit *et al.,* 2010; Nwaoguikpe, 2010; Farombi and Owoeye, 2011; Igwe *et al.*, 2015). Normally, V. amygdalina does not produce seeds but its cultivation is usually done by stem planting and mostly grown in tropical areas. This plant is majorly found in domestic areas and commercial plantation or forest (Yeap *et al*., 2020).

2.2 ETHNOMEDICINAL uses of *Vernonia amygdalina*

Vernonia amygdalina are called different local names which vary from country to country. The bitter taste had been associated with the presence of saponins, alkaloids, tannins, and glycosides. These made them act as a bittering agent and a hop substitute used for controlling microbial contamination in beer brewing without reducing the quality of malt (Ankit *et al*., 2010; 2011; Farombi and Owoeye, 2011).

Croton hirtus can be harvested twice per month for the period of seven years. They are popularly used for food and traditional medicine, their characteristic odour and bitter taste can be reduced either by washing in several changes of water or by boiling before consumption (Nwaoguikpe, 2010 ;). Plate 2 below is representing *Vernonia amygdalina*De leaf.

Plate 2.1: ***Vernonia amygdalina*****Del** **Photo credit: Joy Abgor**

2.3 Description of Croton hirtus L'her.

Croton hirtus is a tropical plant of the genus Croton of the family Euphorbiaceae. It is commonly called rushfoil,*Croton hirtus* is an erect plants mostly trees and herbs; leaves are not lobed or rarely slightly so, serrate, the larger ones sometimes doubly crenate-serrate, 2.5-7 cm. long, 1-4.5 cm broad, with a pair of long-stipitate glands at the base; racemes up to 3 cm. long stem hirsute, densely covered with stellate hairs of which one or more rays are longer and more or less erect or deflexed, the others much shorter and appressed; flowers monoecious; racemes up to 5 cm. long, lower part with female flowers, upper part with the much smaller males; leaves ovate, stellate-puberscent beneath, upper surface pilose with stellate hairs with only one long ray (Kilani *et al.*, 2019). Euphorbiaceae is a large family of about 300 generals comprising 7,500 species distributed in five sub families which were originally Alcalyphoidae, Crotonoideae, Euphorbiodeae, and old field Diodeae. It was revealed that the crotonoideae family comprises of 1300 species. Euphorbiaceae is a large family of about 300 generals comprising 7,500 species distributed in five sub families which were originally Alcalyphoidae, Crotonoideae. (Vanwyk *et al.,* 2018).

2.3.1 Ethnomedicinal uses of *Croton hirtus* Linn

The ethnomedicinal uses of ***Croton hirtus*** as anti-plasmodial, antibacterial, antifungal and management of pregnancy problems, eye disease, purgative, and the used of the root for treatment of threatened abortion and hiccups was revealed by the works of (Salatino *et al.,* 2017; Schmelzer and Gurib-fakim, 2018). The biological properties of plants depend on the presence of some chemical constituent; these chemical constituents are often responsible for their physiological actions. Plants to protect itself produce Phytochemical, but current research demonstrate that they also have the ability to protect humans and animals against diseases. The plant, *Croton hirtus* was found to possess some biological properties as well as ethno medicinal uses, which were revealed from the works of (Kilani *et al.*, 2019; Selowa *et al.*, 2019) that the plant was active as antimicrobial and anti-parasitic agents, active as antiulcer and anticancer agents.

Plate 2.4: ***Croton hirtus*** **Linn** **Photocredit: Joy Agbor**

2.3.2 Description of *Carica papaya* Lindl

Carica papaya is a dicotyledonous, polygamous and diploid species and is commonly called pawpaw (Agarwal *et al*., 2016). The papaya is a large perennial herb with a rapid growth rate. The plants are male or hermaphrodite or female (Sharma *et al*., 2013). The papaya plant has an erect branchless, succulent and soft wooded trunk 6-20 feet (1.8-6.1 m) tall, and a palm like head of foliage at the top. They never develop true bark and the trunk marked scars from previous leaf stems and contains an acrid milky latex sap, the leaves are deeply incised and lobed (Adiaha and Adiaha, 2017). Mature leaves are palmate with deep lobes and are supported by smooth and hollow petiole. The leaf scars given by petiole enlarge as the plant grows in circumference (Nugroho *et al.*, 2017). Fruits hang on short stalks in clusters directly from the trunk beneath the umbrella of giant leaves. Papayas' flowers and fruit simultaneously throughout the year. The fruit is ripe when it feels soft (like a ripe avocado or a bit softer) and its skin has attained amber to orange hue. The melon-like fruit varies in size and shape (Roshan *et al.,* 2019). The fruit is oval to round nearly pyri form or elongated club shaped, 15-50 cm long and 10-20 cm thick and 9 kg weight, semi wild (naturalized) plants possess 2.5 to 15 cm length, fruit skin is waxy thin and tough. Immature fruit which is green in colour contain white latex in more amount on ripening process fruits skin starts turning yellow- orange colour to red, becomes aromatic, juicy and sweet (Maisarah *et al.*, 2019). The fruit hangs from short, thick peduncles at the leaf axil (Roshan *et al.,* 2019).

The changed in outer colour of the skin of fruit is an indicator of ripeness, and this change is considered mainly due to an increase in the carotene content and a decrease in chlorophyll. Red flesh colour indicated the lycopene content in fruit (Maisarah *et al.,* 2019). The botanical name of pawpaw, is *Carica papaya,* which is a lozenge tropical fruit, frequently seen in orange-red, yellow-green and yellow-orange hues, with a rich orange pulp. The fruit is not just delicious and healthy, but whole plant parts, fruit, roots, bark, peel, seeds and pulp are also known to have medicinal properties. The many benefits of papaya owed due to high content of Vitamins A, B and C, proteolytic enzymes like papain and chymopapain which have antiviral, antifungal and antibacterial properties.

2.3.3 Ethnomedicinal Uses of Carica papaya Lindl

Papain, a protein is present in unripe fruit which aids in digestion of proteineous materials in food both in acidic, alkaline and neutral medium. Papaya fruit is of high nutritive value and it has low calories of (32 kcal/100 g of ripe fruit). It is a berry type fruit with parietal plancentation (Noshad and Anjum, 2018). *Papaya* possesses anthelmintic, anti-protozoan, antibacterial, antifungal, antiviral, anti-inflammatory, free-radical scavenging, anti-sickling, neuroprotective, diuretic, abortifacient, hypoglycemic and hypolipidemic, antihypertensive, wound healing, antitumor and antifertility activities.

The ripe papaya is used as topical ulcer dressings to promote granulation, healing, and reducing odour in chronic skin ulcers. Green papaya is used for malaria, hypertension, diabetes mellitus, jaundice, intestinal helminthiasis (Maisarah *et al.*, 2019). It also acts as anti-cancer, stroke prevention and blood cholesterol control (Arliana *et al.,* 2015). *Carica papaya* contains phytochemicals including polysaccharides, vitamins, minerals, enzymes, proteins, alkaloids, glycosides, fats and oils, lectins, saponins, flavonoids, and sterols. It is also used in pies, sherbet and salads preparation, jellies, jams, drinks, ice creams as a dried and crystallized fruit. *Papaya* is a good source of in Vitamin C, Vitamin A, Calcium, Riboflavin, Folate, Calcium, Thiamine, Iron, Niacin, Potassium and Fibre (Adiaha and Adiaha, 2017). It is also used in tenderizing meat. *Papaya* also increases the absorption of iron in human biological system *Papaya* fruit contain an alkaloid carpaine having depressant action on the heart (Adiaha and Adiaha, 2017). Flowers are used to treat jaundice and have Emmengogue, febrifuge and pectoral properties (Roshan, 2019; Sharma *et al*., 2019). Research done on papaya flower is less, male flowers are checked for their potential to act as herbal tea which acts as a dietary supplement for consumption (Bergonio and Perez, 2016). The phytochemical of *C. papaya* peel extracts can be well utilized for preparing biocides or insecticidal formulation. Peel of *C. papaya* might be the reason for its larvicidal activity against Aedes aegypti (Hayatie *et al.*, 2015).

Papaya peel too possesses wound healing properties (Parni and Verma, 2019). The leaves are used for colic, fever, beriberi, abortion, asthma in India, and cancer in Australia papaya leaves are used traditionally in treatments like jaundice, malaria, dengue immunomodulatory and antiviral activity (Bergonio and Perez, 2016). Young leaves are rich in flavonoids (kaempferol and myricetin), alkaloids (carpaine, pseudocarpaine, dehydrocarpaine I and II), phenolic compounds (ferulic acid, caffeic acid, chlorogenic acid), the cynogenetic compounds (benzylglucosinolate).They have medicinal properties like anti-inflammatory hypoglycaemic, anti-fertility, abortifacient, and hepatoprotective, wound healing, antihypertensive and antitumor activities (Yogiraj *et al.,* 2019). Young leaves are used in cooking and eaten like spinach in East Indies. Papaya leaves are used as a tonic for heart disease, treatment for stomach ache, and have antioxidant, anticancer, antiseptic and analgesic.

Plate 2.6: ***Carica papaya*** **Lindl** **Photo credit: Joy Agbor**

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection of Plant samples, Identification and Authentication

The plant samples of the polyherbal of Macedar (*Carica papaya* tree bark, *Vernonia amygdalina* Del leaf and *Croton hirtus* leaf) was collected from capitol behind University of Benin, Benin city.

3.2 Drying of the Plant

The leaves of *Carica papaya* tree bark, *Vernonia amygdalina* Del leaf and *Croton hirtus* leaf

Were cut to smaller pieces and air dried at room temperature while pawpaw leaves was as well processed for 7 days. After 7 days of air drying, the samples were placed into an oven (model: COV-8320-C) for 72 hours at 45ºC. The dried sample was crushed into fine powder using an electronic blender (model: Silver Crest SC-1589). The fine powder was placed in an air tight container for further analysis (Obaro *et al*., 2024).

3.3 Extraction Process Using Cold Maceration Method

The powdered sample of the respective plants were weighed 166.67g each to make up 500g ratio of 1; 1; 1 sample and 500 g of pawpaw leaves powder was weighed separately in another jar in a was weighed and placed in another glass jar and 2.5 litres of methanol solvent was added to each of the glass jar containing the respective plant samples.

The mixtures and pawpaw leaves only were macerated with spatulas, then covered tightly and shaken vigorously and frequently. The container with the sample were then kept in a dark cupboard for 72 hours. Keeping the mixture and pawpaw leaves in a dark cupboard was done to prevent distortion and damage of the phytochemicals present. The polyherbal formulation was brought out of the dark cupboard, shaked and poured into cheese clothes over a bowl to filter. The shaft was macerated and squeezed out leaving behind the concentrates. The concentrates were poured into crucibles and placed in a water bath and concentrated at 45^0C for 24 and 72 hours respectively. The concentrates was completely dried in the oven at 45^0C for another 24 and 72 hours respectively. The dried extracts were stored in air tight containers and placed in the refrigerator (model: TR-131L) for further use.

3.4 Anti-bacterial Assay

Anti-bacterial assay was studied using the methods described by Pâmela *et al*. (2012). Pathogenic bacteria cultured for twenty-four hours comprising of gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) and gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) bacteria were used for the *in-vitro* antibacterial assay. All micro-organisms were obtained from the laboratory stock of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, and University of Benin.Antimicrobial agents: pefloxacin 5µg/ml, clotrimazole cream 1mg/ml were used as standard reference drugs.

3.5 Procedure for Antimicrobial Activity Determination

Overnight broth cultures were used to obtain 0.5 standards of bacterium which were used to seed sterile Mollen nutrient agar medium maintained at 45^0 C. Sabour and dextrose agar medium were similarly seeded with fungi. Seven holes (6 mm) respectively were bored in each of the plates (9 cm diameter) with an aseptic corn borer, when seeded plate had solidified 500, 200, 100, 50, 25, 12.5 and 6.5 mg/ml of the extract was prepared with distilled water by preparing a stock solution and carrying out double-fold dilutions on it. With the aid of a syringe, the wells were filled with 0.25 ml (5 drops) of different dilutions of the extract while the centre wells were filled with 20 ug/ml of standard drug. Diameters of zones of inhibition were determined after incubating plates at 37^o C for 24 hours. This was done for the two (2) different extracts.

3.6 DATA ANALYSIS

Results from the studies were taken as the Mean ± SEM. Statistical analysis was arrived at using graph pad prism 8 version software (UK). Comparisons amongst treated and control groups were analysed using one-way ANOVA by, Dunnett's multiple comparisons test. $P < 0.05$ was regarded as indicating significant differences.

CHAPTER FOUR

4.0 RESULT

4.1 Effect of The comparative polyherbal formulation of Macedar and pawpaw leaves on Antibacterial assay

The comparative antibacterial effect of Macedar and Pawpaw leaves *was* carried out using both gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) as well as gram-negative (*Pseudomonas aeruginosa and Escherichia coli*).

The administration of doses of the extracts of Macedar and pawpaw leaves extracts were (500, 250, 125 and 62.5 mg/ml) exhibited dose-dependent increases in the zone of inhibition, which were significant (*= $p \leq 0.05$ **= $p \leq 0.01$; ***= $p \leq 0.001$). The minimum and maximum zones of inhibitions were observed at 6.0±0.1and 36.20±4.0*** at 500 mg/ml respectively (Table 4.1-Table 4.4).

4.1.1 Comparative antimicrobial Effect of Methanol Extracts of Macedar and *Carica papaya* leaves

The maximum inhibitory concentrations for **Macedar** and *Carica papaya* leaves extracts were observed at 500 mg/ml. the highest maximum inhibitory value (36.20±1.6) compared to methanol extract of pawpaw leaves (31.3±1.9). Although both extracts were significant at (*=

p≤0.05 (125 mg/ml); **= *p≤0.01* (250 mg/ml)*;* and ***= *p≤0.001* (500 mg/ml)) when compared with the minimum inhibitory concentration (62.5 mg/ml).

Table 4.1: Comparative Antimicrobial Effect of the Methanol Extracts of Macedar and *caricapapaya leaves* on *Pseudomonas aeruginosa*

CHAPTER FIVE

5.0 DISCUSSION

.CONCLUSION

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APPENDIX I

TABLE 1.0 : Methanol extract against *Pseudomonas aeruginosa*

METHANOL (mg/ml)	500	250	125	62.5	31.25
PLATE 1	25mm	14mm	6mm	NA	NA
PLATE 2	24mm	12mm	10mm	5mm	NA
PLATE 3	22mm	16mm	8mm	4mm	NA

TABLE 2.0 : Aqueous extract against *Pseudomonas aeruginosa*

AQUEOUS EXTRACT (mg/ml)	500	250	125	62.5	31.25
PLATE 1	17mm	10mm	8mm	NA	NA
PLATE 2	20mm	15mm	10mm	NA	NA
PLATE 3	18mm	13mm	6mm	NA	NA

TABLE 3.0: Hot water extract against *Pseudomonas aeruginosa*

HOT WATER (mg/ml)	500	250	125	62.5	31.25
PLATE 1	30mm	17mm	12mm	NA	NA
PLATE 2	27mm	20mm	8mm	NA	NA
PLATE 3	26mm	21mm	6mm	NA	NA

TABLE 4.0: Ethanol extract against *Pseudomonas aeruginosa*

ETHANOL (mg/ml)	500	250	125	62.5	31.25
PLATE 1	26mm	19mm	11mm	NA	NA
PLATE 2	31mm	14mm	7mm	NA	NA
PLATE 3	28mm	15mm	7mm	NA	NA

TABLE 5.0: Methanol extract against *Staphylococcus aureus*

METHANOL (mg/ml)	500	250	125	62.5	31.25
PLATE 1	20mm	18mm	6mm	NA	NA
PLATE 2	28mm	16mm	12mm	NA	NA
PLATE 3	26mm	14mm	10mm	NA	NA

TABLE 6.0: Cold water extract against *Staphylococcus aureus*

COLD WATER (mg/ml)	500	250	125	62.5	31.25
PLATE 1	31mm	25mm	12mm	NA	NA
PLATE 2	33mm	28mm	10mm	NA	NA
PLATE 3	31mm	24mm	8mm	NA	NA

TABLE 7.0: Hot water extract against *Staphylococcus aureus*

HOT WATER (mg/ml)	500	250	125	62.5	31.25
PLATE 1	28mm	11mm	NA	NA	NA
PLATE 2	27mm	13mm	NA	NA	NA
PLATE 3	29mm	12mm	NA	NA	NA

TABLE 8.0: Ethanol extract against *Staphylococcus aureus*

ETHANOL (mg/ml)	500	250	125	62.5	31.25
PLATE 1	26mm	19mm	8mm	NA	NA
PLATE 2	27mm	17mm	10mm	NA	NA
PLATE 3	29mm	14mm	7mm	NA	NA

TABLE 9.0: Methanol extract against *Bacillus cereus*

METHANOL (mg/ml)	500	250	125	62.5	31.25
PLATE 1	16mm	15mm	3mm	NA	NA
PLATE 2	22mm	14mm	8mm	NA	NA
PLATE 3	21mm	16mm	5mm	NA	NA

TABLE 10.0: Cold water extract against *Bacillus cereus*

Methanol Extract	500	250	125	62.5	31.25
PLATE 1	16mm	9mm	NA	NA	NA
PLATE 2	21mm	11m	NA	NA	NA
PLATE 3	17mm	7mm	NA	NA	

TABLE 11.0: Hot water extract against *Bacillus cereus*

Aqueous Extract (mg/ml)	500	250	125	62.5	31.25
PLATE 1	29mm	14mm	9mm	NA	NA
PLATE 2	26mm	18mm	7mm	NA	NA

PLATE 3	27mm	12mm	8mm	NA	NA

TABLE 12.0: Ethanol extracts against *Bacillus cereus*

METHANOL (mg/ml)	500	250	125	62.5	31.25
PLATE 1	26mm	12mm	4mm	NA	NA
PLATE 2	28mm	11mm	6mm	NA	NA
PLATE 3	26mm	10mm	4mm	NA	NA

APPENDIX II
MÜLLER-HINTON AGAR

- Beef extract: 2g
- Casein hydrolysate: 17.5g
- Starch: 1.5g
- Agar: 17.0g

Dissolved in 1 liter of distilled water

CIPROFLOXACIN

- Croscarmellose sodium
- Microcrystalline cellulose

- Povidone
- Magnesium stearate