IN VITRO ANTI-MICROBIAL EVALUATION OF DIFFERENT CRUDE EXTRACTS FROM *DILLENIA PENTAGYNA* LEAF AND STEM

Dissertation
Submitted in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy

Submitted by

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JULY, 2012

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation, entitled

'In Vitro Anti-Microbial Assessment of Different Crude Extracts from *Dillenia pentagyna* Leaf and Stem' is an authentic and genuine research work carried out by me under the guidance of Ms. Nazia Hoque, lecturer, Department of Pharmacy, East West University, Dhaka.

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CERTIFICATE BY THE INVIGILATOR

This is certify that the dissertation, entitled

In Vitro Anti-Microbial Assessment of Different Crude Extracts from *Dillenia pentagyna* Leaf and Stem' is a bonafide research work done by Ridwana Akhand, in partial fulfillment of the requirements for the Degree of Bachelor of Pharmacy.

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'In Vitro Anti-Microbial Assessment of Different Crude Extracts from *Dillenia pentagyna* Leaf and Stem' is a bonafide research work done by Ridwana Akhand, under the guidance of Ms. Nazia Hoque, lecturer, Department of Pharmacy, East West University, Dhaka.

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ABSTRACT

Background: *Dillenia pentagyna* is widely distributed in tropical regions of Asia. The traditional use of this plant suggests its possible antimicrobial properties, but its efficacy has not been examined yet. ***Objective:*** The purpose of this research was to evaluate the antimicrobial efficacy of crude extracts of *Dillenia pentagyna* leaf and stem against pathogenic microorganism using the disk diffusion method. . In this study, the crude ethyl acetate, ethanol & water extract of both leaf and stem was assessed. ***Materials and methods:*** Two different concentrations of 800µg/disc and 400µg/disc for crude leaf and stem extracts of *Dillenia pentagyna* were tested for antimicrobial efficacy against five Gram-positive (*Bacillus sereus, Bacillus subtilis, Bacillius megaterium, Staphylococcus aureus & Sarcina lutea),* eight Gram negative bacteria(*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Salmonella paratyphi, Shigella dysenteriae, Shigella boydii, Vibrio mimicus & Vibrio parahemolyticus)* and two fungi (*Candida albicans & Saccharomyces cerevisiae).* The antimicrobial activity of the extracts was observed against the standard antimicrobial agent Cephradine of 30 µg/disc. The sensitivity of plant was tested using the disk diffusion method by observing and measuring the zone of inhibition for each sample against each microorganism. ***Results:*** Maximum activity was revealed by ethyl acetate extract of the stem followed by the ethanol extract. The ethyl acetate and ethanol extract of leaf showed more or less sensitivity almost against all pathogens. The water extracts revealed very less activity against the microorganisms. ***Conclusion:*** This plant may be effective for treatment of different pathogenic diseases.

Keywords: *Dillenia pentagyna,* antimicrobial activity, microorganism, disk diffusion, zone of inhibition.

Chapter One

Introduction

INTRODUCTION

1.1 PREFACE

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. One way to prevent antimicrobial resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents [1].

Clinical microbiologists have great interest in screening of medicinal plants for antimicrobial activities and phytochemicals as potential new therapeutics. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties. Naturally occurring combinations of these compounds can be synergistic and often results in crude extracts having greater antimicrobial activity than the purified individual constituents. For these reasons, medicinal plants are important substances for the study of their traditional uses through the verification of pharmacological effects and can be natural composite sources that act as new ant infectious agents [2].

The plant chosen for the present study is *Dillenia Pentagyna* belonging to the family *Dilleniaceae* which is a deciduous tree found mainly in rain forests, thickets and hills below 400 m. Traditionally the plant was patronized for its unique property of healing all type of cuts, wounds and boils in very less time and with less pain. According to Ayurveda, the plant is used in anal fistula, wounds, diabetes, diabetic carbuncle, neuritis, pleurisy, pneumonia, and burning sensation [3]. The tribal and folk communities also use various parts of it in different ailments and diseases like delivery, bone fracture, body pain, piles, diarrhoea and dysentery. Literature survey revealed that the leaves of the plant have been evaluated for anti cancer effects. phytochemical studies on *Dillenia Pentagyna* included characterization of tanins, triterpenoids, saponins, flavanoids and glycosides [4].

This study was conducted to address the antimicrobial activity of *Dillenia Pentagyna* against some pathogenic bacteria and fungi when extracted the leaf and stem parts in different solvents.

1.2 ANTIBIOTIC RESISTANCE BY MICROORGANISMS

The continuously emergence of new antibiotic resistance strains day by day have become the problems among the people. Microorganisms are always having the ability to protect themselves against naturally antibiotics by showing resistancy by exchanging the genetic material with other organisms. They acquire and adapt properties of other organisms through this genetic exchange into their own genetic material to gain new resistancy. However, the problems of antibiotic resistance by the microorganisms become worse as the microorganisms also found to be resistance against the synthetically synthesize antibiotics. Thus, alternative methods to fight against this resistance microorganisms are needed and researchers have struggled to find new antibiotic that have the capability to inhibit the microorganisms [1].

1.3 ANTIMICROBIAL PROPERTIES OF MEDICINAL PLANTS

Even though there are numerous drugs produce in the market, the evolution of microorganisms has caused the microorganisms to become resistance towards the antibiotic. Hence, new drugs need to be produced in order to fights against the microorganisms. For centuries, plants have often being used and act as valuable natural resources that help to maintain human health. Almost 80% of world populations in developed countries use traditional medicine derived mostly from medicinal plants [2]. Many medicinal plants have been discovered everyday around the world. The demands for medicinal plants are rapidly increasing not only in developed country but also in developing countries as well. The pressure for the utilization of useful compounds in medicinal plants as new therapeutic drugs from the also increase due to this matter. The medicinal plants are very important because of their antimicrobial properties and have been focused by many researchers because of the bioactive compound present in their secondary metabolites products [2].

1.4 PLANT PROFILE: *DILLENIA PENTAGYNA*

Dillenia Pentagyna is a flowering plant belongs to the family Dilleniaceae. The genus is named after the German Botanist Johann Jacob Dillenius, and consists of evergreen or semi-evergreen trees and shrubs. The species name is derived from the Greek pente (five) and gyne (woman, ovary) [5].

1.4.1 Scientific Classification of Dillenia Pentagyna **Roxb.** [6]

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopside

Order: Dilleniales

Family: Dilleniaceae

Genus: *Dillenia*

Species: *Dillenia Pentagyna*

1.4.2 Vernacular Names

Dillenia Pentagyna is known under different vernacular or local names throughout the regions it is distributed [7]. Some common local names are:

English: Five-carpelled simpoh, Nepalese elephant apple

Sanskrit: Aksikiphal, Punnaga

Bengali: Ajugi, Ajuli, Hargaza etc.

Hindi: Karmal

Assamese: Okshi

Oriya: Railgatcho

Chinese: Xiao hua wu ya guo.

Nepalese: Agaaai, Taatarii.

Thai: San chang

Cambodian: Pheng

Myanmar: Zinbyun

Java: Janti, Sempu

1.4.3 Geographical Distribution

Dillenia Pentagyna is mainly distributed throughout the lowland evergreen broadleaf rain forests (tropical) of China, India, Malaysia, Myanmar, Thailand, Bhutan, Indonesia, Nepal, and Vietnam [6]. In Bangladesh it is found in forests of Chittagong, Chittagong Hill Tracts, Cox's Bazar, Dhaka, Tangail and Sylhet [3].

Figure 1.1: Geographical Distribution of *Dillenia Pentagyna.*

1.4.4 Plant Description

A deciduous tree is 15 m tall or more with ascending branches and fluted stem. Bark is smooth, grayish white and peeling off in to flakes. Twigs are glabrous, thick, dry and dark brown [9].

Figure 1.2: *Dillenia Pentagyna* Plant

The leaves thinly coriaceous, oblong or obovate oblong, clustered at twig ends, 20-60 cm long and 10-25 cm wide; apex is slightly acute or obtuse. Base is narrowed and often decurrent into wings; petiole is 2-5 cm, glabrous, with narrow wings. Lateral veins are 32-60 pairs, or more and very prominent. The leaves are shed in January-February [9].

Figure 1.3: *Dillenia Pentagyna* Leaf

Flowers are about 4 cm across, appearing profusely in fascicles of 3-8 from leafless branches. Pedicels are glabrous and bracteoles are caduceus. Sepals are green, oval-shaped; size is not equal, 8-12 mm long and 5-9 mm wide. Petals are yellow, long-obovate, 1.5-2 cm long, 8 mm wide. The flowers appear along the leafless branches in March-April [10].

Figure 1.4: *Dillenia Pentagyna* Flower

Carpels are 5 or 6 in number, 3.5-4 mm in diam.; each carpel contains ovules 5-20. Pseudocarp is indehiscent, yellow, orange, or red; mature carpel is 1(or 2)-seeded. Fruits are globose, 0.5-2 cm across, very acidic. Ripe fruit is red and yellow. The fruits ripen in May-June. Seeds are ovoid, 5 mm long, 3.5 mm wide, black and aril [9, 10].

Figure 1.5: *Dillenia Pentagyna* Fruit

1.4.5 Medicinal Uses

Dillenia Pentagyna is used in tuberculosis, fistula, sores, carbuncle, neuralgia, pleurisy and pneumonia. It is used as a cold remedy too [3]. According to Ayurveda, the plant is also used

in wounds, diabetes, and burning sensation. Tribal and folk communities use different parts of this plant in different medical purposes. For example, barks and leaves are used for the treatment of diarrhoea and dysentery in Rema-Kalenga. Bark is used for blood dysentery in Khagrachari. Many folk communities in India use bark, leaf and root of *Dillenia Pentagyna* in delivery, bone fracture, piles and body pain [3]. Different pharmacological studies showed that *Dillenia pentagyna* could be a potent anti cancer drug. Antimicrobial activity of the plant is also observed in studies.

1.5 ANTIMICROBIAL SUSCEPTIBILITY TEST

The sensitivity of the microorganisms towards the antimicrobial agent can be tested using the antimicrobial susceptibility test. The most commonly used antimicrobial susceptibility test is disc diffusion method [11].

1.5.1Disk Diffusion Method

The disc diffusion method, a much simpler method compared to the other methods. The basic principle of disc diffusion test is by placing a disc impregnated with extract solution onto the surface of agar swab with bacterial inoculums. After incubation overnight, the antimicrobial activity was defined by measuring diameter of inhibition zone around the discs [11]. The discs were impregnated with different concentration of plant extract solution. The drugs will diffuse from the discs into the agar. The farther the drugs diffuse from the discs, the lower its concentration. Inhibition zone produces shows the sensitivity of the bacterial towards the chemotherapeutic agents. Larger inhibition zone, the more sensitive the bacteria will be [11].

1.6 OBJECTIVE OF THE RESEARCH

The objective of this research is to determine the antimicrobial activity of the crude extracts using disk diffusion technique and also identify the possibilities of using *Dillenia pentagyna* extracts for an antimicrobial drugs based on its antimicrobial activity.

Chapter Two

Literature Review

REVIEW OF LITERATURE

2.1 ETHNOMEDICINAL STUDY

The bark of *Dillenia pentagyna* is used by tribals and folklore traditions in India for treatment of cuts and burns [12].

The seed and the bark of *Dillenia pentagyna* are used against cancer by the Koch-Rajbanshi people of western Assam [13].

Fruit juice mixed with sugar and water is used as cooling beverage in fever and cough among Malamalasar tribe of Palakkad district of Kerala state [14].

Bark of *Dillenia pentagyna* is used as digestive in the village of Amboli and an in the Western Ghats of India [15].

The plant is also used in the treatment of gynecological disorders by the tribal of Madhya Pradesh. Tree gum is used for easy delivery and reduces uterine complications [16].

The plant part is used as anticancer & antiulcer agent; decoction of the bark is taken orally for diabetes, paste of bark is applied externally on rheumatic pains in the tribal area of Mizoram. Aerial part and bark decoction is taken orally for diabetes, the paste of aerial part and bark is applied externally on rheumatic pains and the wood is used for anticancer & antiulcer agents in Mizoram [17].

One to two inches of the stem bark from *Dillenia pentagyna* are crushed with a pinch of common salt. This extract is administered once daily for three days to treat cough in the Eastern Ghats region of Andhra Pradesh. Fruits from this species are laxative and used in abdominal pains by the folk of Khammam district of Andhra Pradesh [18].

The tribes of nasik district in Maharashtra use the stem bark of the plant for the ailment of jaundice and urinary complaints by mixing the extract in water through crushing and squeezing. This preparation is used once daily till cure. To cure the wound, bark of Karmal (*Dillenia pentagyna*) tree (25-50 gm) was lavigated in water and the paste formed was applied on the wound for 3-5 days by the village people in Konkan region of Maharashtra [19].

5ml of fruit decoction of *Dillenia pentagyna* is mixed with 3g rhizome paste of *Zingiber montanum* and the mixture is taken thrice a day for three days before food against blood dysentery by the tribals of the Deogarh district. Stem Bark of the plant locally known as Raayi is used in easy Delivery and blood Dysentery by natives of Bargarh district of Orissa in India [20]. Decoction of stem bark (2ml) mixed with lime water (1ml) is taken twice a day before food for cure of blood dysentery and Midwives (Dai) of ethnic group uses tree gum for delivery purpose. These ailments are available among Santal, Bathudi and Kolha tribes of Mayurbhanj district,

around Similipal bioreserve, Orissa. Flowers of *Dillenia pentagyna* are eaten as vegetable by the people of Malkangiri district of Orissa [21].

2.2 PHYTOCHEMICAL STUDY

In a study the freshly prepared extracts of *Dillenia pentagyna* were chemically tested and the presence of chemical constituents such as alkaloids, tannins, saponins, flavonoids and iridoids were identified [22].

A comparative analysis of *Dillenia pentagyna* demonstrated presence of fatty mater, calcium oxalate and tannin [23].

Some other studies reported that *Dillenia pentagyna* is a useful source of tannins. The stem contains about 6% of tannin. The bark and leaf also contain some amount of tannin [24]. Tannins are good source of antimicrobial agents Stem contains α-*L*-rhamnopyranosyl-3-OH-lup-20(29)-en-28-oic acid [2].

Stem contains α-*L*-rhamnopyranosyl-3-OH-lup-20(29)-en-28-oic acid [25].

Two new flavonoid glycosides, naringenin 7-galactosyl(1 → 4)glucoside and dihydroquercetin 5- galactoside, have been characterized from stem tissue of *Dillenia pentagyna*. Rhamnetin 3-glucoside was also isolated from the plant [27]. The other flavonoids those has been isolated from the plant are taxifolin 5-galactoside and 5,7,4'-trihydroxyflavanone 7-O-galactosylglucoside[26].

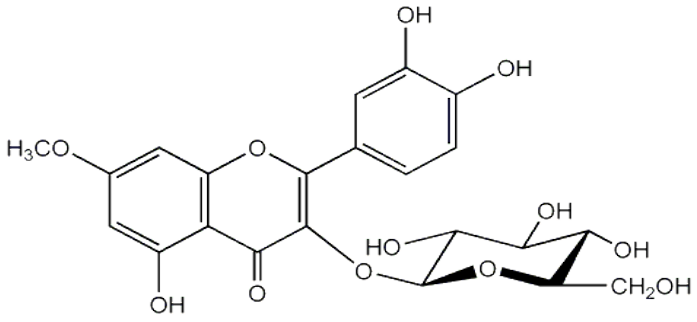


Figure 2.1: Rhamnetin 3-glucoside

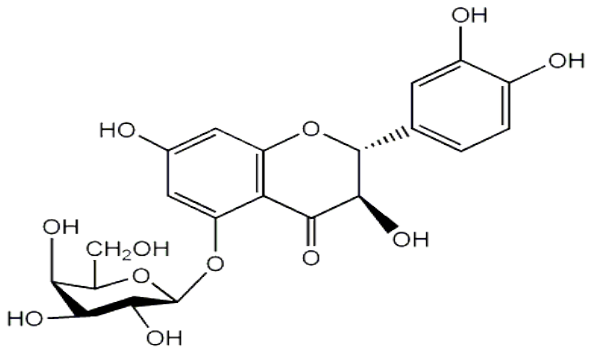


Figure 2.2: Taxifolin 5-galactoside

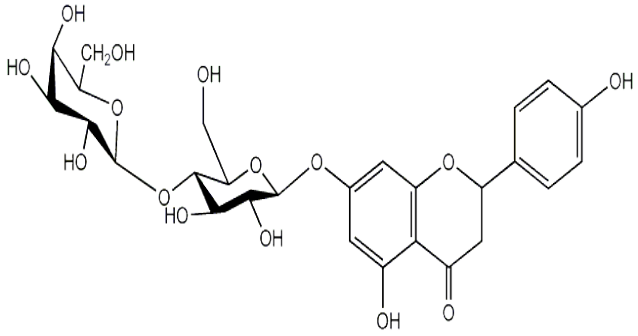


Figure 2.3: 5,7,4'-Trihydroxyflavanone 7-O-galactosylglucoside

It has been reported that bark and stem of the plant contains lupeol, betulin, betulinic acid and morolic acids [27].

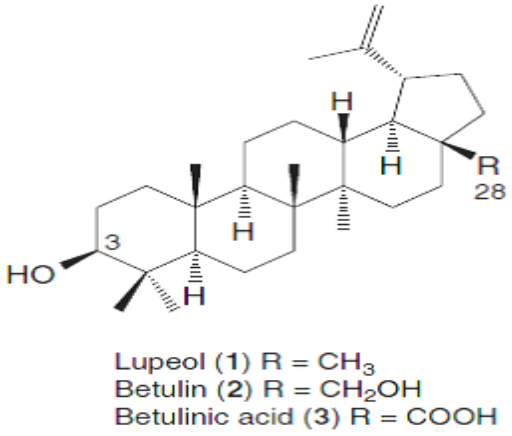


Figure 2.4: Lupeol, Betulin and Betulinic acid

2.3 PHARMACOLOGICAL STUDY

Dillenia pentagyna were studied for their antitumour activity against murine ascites Dalton's lymphoma (DL) in vivo. From the study it may be concluded that the natural product of D. pentagyna promises to be active against Dalton's lymphoma and the decrease in glutathione level may be one of the important steps in resulting this antitumour effect [28].

In another *D. pentagyna* extract mediated decrease in the lipid peroxidation in the tissues of tumor bearing mice indicated its possible protective function against tissue damage caused by oxidative stress in tumorous condition. Decrease in sialic acid content in the tissues of tumor-bearing mice and paricularly in the tumor cells after this plant extract treatment may also help in facilitating / increasing host survivability [29.30].

The ethanol extract of D. Pentagyna showed the most potent antitumor activity, i.e. % ILS ~ 55% and % ILS ~ 48% at a dose of 50 and 100 mg/kg/day compare to other plants of the study [31].

Dillenia pentagyna showed little activity (5 to 8mm) against all pathogenic microorganisms of a study in which evaluation of antimicrobial activity of different plant was studied [32].

Chapter Three

Materials & Methods

MATERIALS & METHODS

3.1 MATERIALS

3.1.1 Plant Sample

- Ethanol Extract of *Dillenia pentagyna* Leaf
- Water Extract of *Dillenia pentagyna* Leaf
- Ethyl Acetate Extract of *Dillenia pentagyna* Leaf
- Ethanol Extract of *Dillenia pentagyna* Stem
- Water Extract of *Dillenia pentagyna* Stem
- Ethyl Acetate Extract of *Dillenia pentagyna* Stem

3.1.2 Strains of Microorganism

- *Bacillus cereus*
- Bacillus *subtilis*
- *Bacillius megaterium*
- *Staphylococcus aureus*
- *Sarcina lutea*
- *Escherichia coli*
- *Pseudomonas aeruginosa*
- *Salmonella typhi*
- *Salmonella paratyphi*
- *Shigella dysenteriae*
- *Shigella boydii*
- *Vibrio mimicus*
- *Vibrio parahemolyticus*
- *Candida albicans*
- *Saccharomyces cerevisiae*

3.1.3 Culture Media

- Nutrient Agar (Himedia Laboratories, India).

3.1.4 Reagents & Solvents

- Ethanol
- Ethyl Acetate
- Distilled Water
- Normal Saline (9% NaCl)

3.1.5 Apparatus & Equipments

- Screw Cap Test Tube.
- Beaker.
- Electronic Balance (ELH 3000, Shimadzu, Japan).
- Aluminium Foil.
- Spatula.
- Pipette
- Autoclave (HIRAYAMA, Japan).
- Laminar air flow (ESCO, Singapore).
- Hot air oven (FN-500, Niive).
- Incubator (BK 4266).
- Vortex mixer.
- Petri dishes.
- Culture Bottle
- Filter paper (Whatman 40).
- Hole puncher.
- Paper Disk.
- Inoculating Loop.
- Gas Burner.
- Forceps.
- Cotton Buds.
- Micropipette (Eppendrof, Germany).
- Eppendrof micropipette tip.
- Eppendrof tube.
- Measuring Scale.

3.3 METHODS

3.3.1 Collection of Crude Extract and Preparation of Dosage Form

The plant samples were collected from the microbiology lab, Department of pharmacy, East West University. Crude extracts were measured in analytical balance, and dissolved in Water, Ethanol, Ethyl Acetate in a specific concentration 20 µg/ µL and 40 µg/ µL. The liquid form of sample was made for optimal concentration of dose in each paper disk. The disk concentrations used were 400 µg and 800 µg.

3.2.2 Preparation of Bacterial Culture

3.2.2.1 Collection of Microorganism Strains

The fifteen microorganism samples were collected from the laboratory of pharmacy department of East West University. All the strains were cultured from the mother strains as a stock culture in vials, and then they were re-cultured in Petri dishes.

3.2.2.2 Preparation of Agar Media

According to the instructions on the nutrient agar container, 28 gram Nutrient Agar is required to mix in 1000ml of distilled water. So, 8.4gram Nutrient agar was weighed and then 300ml distilled water was added to prepare 300ml Agar solution. This preparation was kept in a 400ml culture bottle.

Figure 3.1: Nutrient Agar in Culture Bottle

3.2.2.3 Sterilization

Petri dishes were autoclaved in the autoclave machine (HIRAYAMA, Japan) at 121°C. After the autoclaving, they were washed by using detergents and then left for drying in the Hot air oven (FN-500, Niive) for 50-60 minutes. Petri dishes and the prepared media were autoclaved for sterilization and placed into laminar air flow (ESCO, Singapore) to prevent contamination.

Figure 3.2: Autoclave machine (HIRAYAMA, Japan) & Hot air oven (FN-500, Niive)

3.2.2.4 Inoculation

After the sterilization by autoclave & hot air oven, sterile Petri dishes and Agar solution glass container were kept under laminar air flow to prevent contamination. The prepared Agar solution was poured into each of the fifteen Petri dishes in a way so that each Petri dish gets 15-20 ml agar medium. Agar medium was dispensed into each Petri dish to gets 3-4mm depth of agar media in each Petri dish. After pouring the agar medium, all Petri dishes were kept in room temperature, so that agar medium can become properly solidified.

Petri dishes were labelled on the bottom. To streak a specimen from a culture tube, metal transfer loops were first sterilized by flaming the wire loop held in the light blue area of a gas burner just above the tip of inner flame of the flame until it is red-hot. The culture containing test

tube cap was removed. The loop was then inserted into the culture tube and a loopful of strain was removed. The cap of the test tube was replaced and put it back into the test tube rack. The lid of the agar plate was opened just sufficiently enough to streak the plate with the inoculation loop. The loop was dragged over the surface of the top of the plate back and forth in a "zig-zag" formation. The loop was removed and the petri dish was closed.

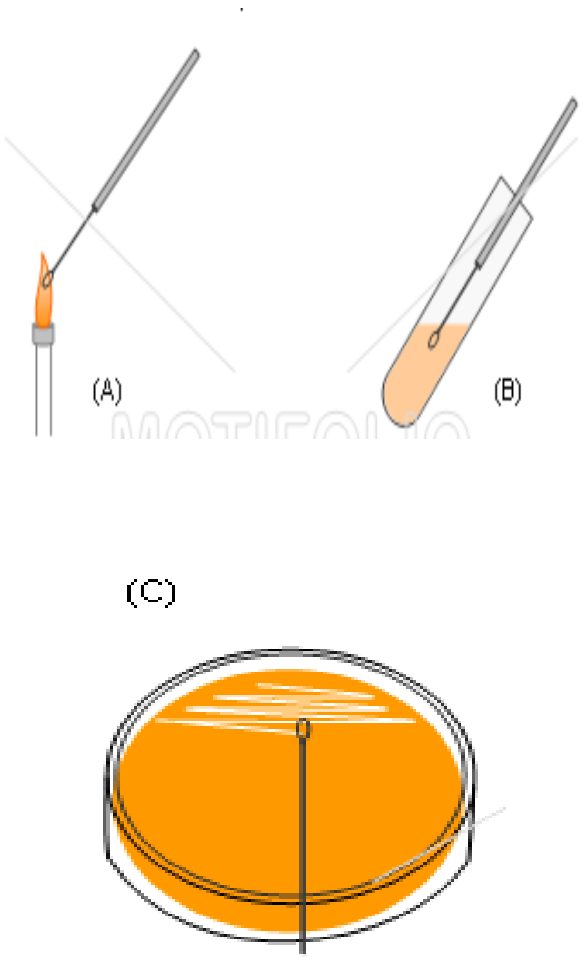


Figure 3.3: Steps of Inoculation

3.2.2.5 Incubation

Then all the prepared agar plates with respective micro organisms were placed inside a bacteriological incubator for 18 hours to allow the growth of pure fresh culture of micro organism in each of the Petri dishes.

Figure 3.4: Incubator

3.2.2.6 Preservation of cultured micro organisms:

After 18 hours incubation, all the Petri dishes with respective micro organism cultures were removed from Incubator and then were kept in a refrigerator for further use in in-vitro antimicrobial test.

3.2.3 *In Vitro* Antimicrobial Screening of Plant Extract

3.2.3.1 Preparation of Filter Paper Disk

Whatman filter paper no. 1 was used to prepare discs approximately 6 mm in diameter by a Hole puncher.

Figure 3.5: Filter paper discs

3.2.3.2 Preparation of Media

Media (Agar) was prepared with distilled water and nutrient agar according to the instruction written on the agar container.

3.2.3.3 Sterilization

Media and the required equipments were sterilized in the Autoclave for 1 hour. Where temperature range was 60°C to 121°C and pressure was 1 atm. After sterilization, all the things *were kept under laminar airflow.*

3.2.3.4 Agar Plate Preparation

Agar media was dispensed into petri dishes to get 3-4mm depth of agar in each dish. This was done under laminar airflow. After pouring the media, petri dishes were kept in room temperature to become properly solidified.

3.2.3.5 Inoculum Preparation

According to the direct colony suspension method, the prepared 0.9% normal saline was poured into eppendrof tubes in a way that each may be able to contain 1ml of saline. These eppendrof tubes were labelled into respective sample micro organism names. Then, micro organism culture plate that was stored in refrigerator was brought. With a sterile loop micro organism colonies from the bacterial culture plate was isolated and dipped into tube containing saline suspension. Then the tube was closed and the micro organism inoculums were mixed properly by a vortex mixer.

Figure 3.6: Eppendrof Tubes & Vortex Mixer

3.2.3.6 Inoculation

Optimally, within 15 minutes after adjusting the turbidity of the inoculums suspension (tube containing suspension of micro organism), a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This helped to remove excess inoculums from the swab.

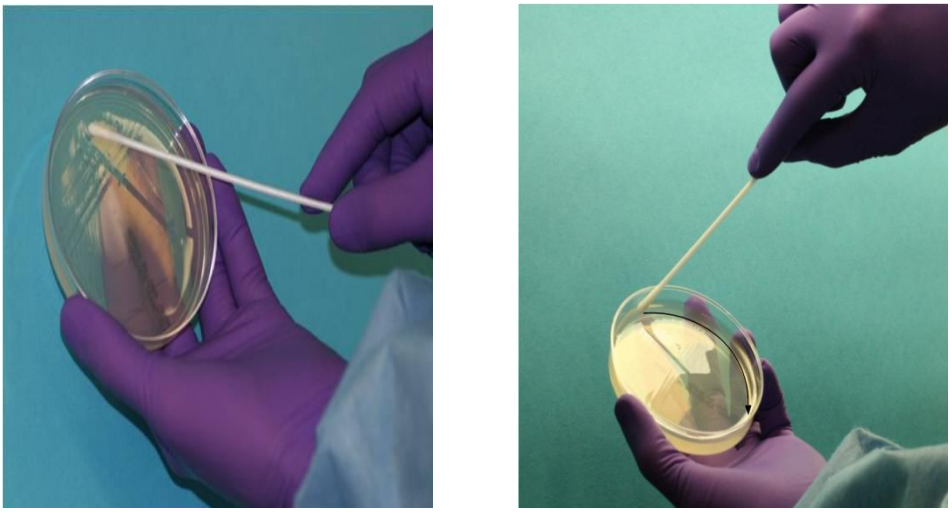


Figure 3.7: Inoculation Process

The dried surface of the agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times , rotating the plate approximately 60º each time to ensure an even distribution of inoculums. As a final step, the rim of the agar was swabbed. This process was performed for each of the fifteen micro organisms. All the plates were labeled with name of micro organisms, dose of the plant disc, standard antibiotic dose at backside.

The lid may be left agar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the plant impregnated disked. An extreme in inoculums density was avoided.

3.2.3.7 Application of Plant Extract to Prepared Disks and Placement of Disks

A 20-200µl micropipette was adjusted to 20µl. Sterile discs of six millimeter width had been impregnated with 20 µl of test extract and introduced onto the upper layer of the seeded agar plate.

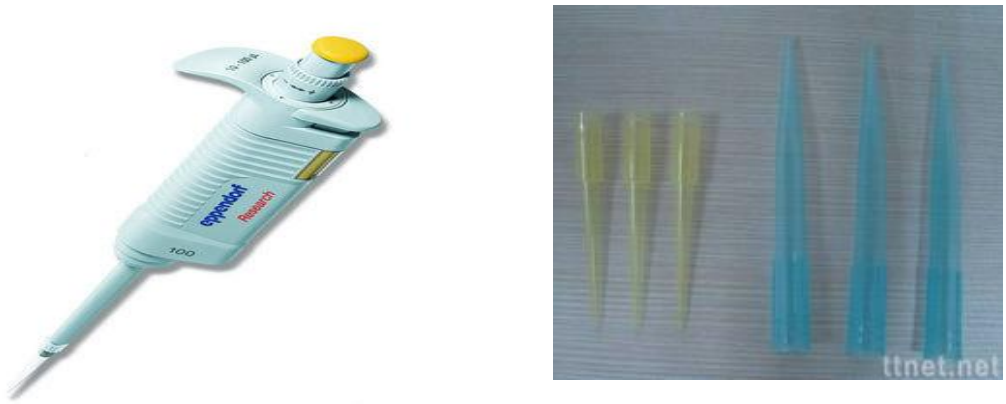


Figure 3.8: Eppendrof Tube & Tips

3.2.3.8 Incubation

The plates were incubated soon after placing the disc. The temperature range of 35°c ±2°c is normally required for incubation and the incubation time was 24 hours which were considered as standard for this test.

3.2.3.8 Measuring zone sizes:

Following incubation, the zone sizes were measured to the nearest millimeter using a ruler. The diameter of the disc was included in the measurement.

The whole process of antimicrobial screening was done two times and the mean values were presented.

Chapter Four

Result & Discussion

RESULTS & DISCUSSION

4.1 RESULTS

The results of the experiment are interpreted into tabular representation and graphical representation. The values of zone of inhibition were placed in the table and from that a graph of zone of inhibition vs. microorganism was plotted for each table. Examples of some results (Figure: 4.5-4.10) are given later in this dissertation.

Table 4. 1: Antimicrobial activity of different solvent extracts of at a *Dillenia pentagyna* stem at a concentration of 800μg/disc.

Name of Microorganism	Zone (mm) of Inhibition by Water Extract	Zone (mm) of Inhibition by Ethanol Extract	Zone (mm) of Inhibition by Ethyl Acetate Extract	Zone (mm) of Inhibition by Cephradine
Bacillus cereus	0	11	14	20
Bacillus subtilis	0	9	15	26
Bacillius megaterium	0	0	12	20
Staphylococcus aureus	0	10	13	26
Sarcina lutea	0	12	22	22
Escherichia coli	0	10	20	23
Pseudomonas aeruginosa	8	17	18	25
Salmonella typhi	0	12	14	20
Salmonella paratyphi	0	0	15	24
Shigella dysenteriae	0	8	9	22
Shigella boydii	0	12	19	30
Vibrio mimicus	0	7	11	20
Vibrio parahemolyticus	0	13	15	22
Candida albicans	0	7	15	20
Saccharomyces cerevisiae	0	14	9	24

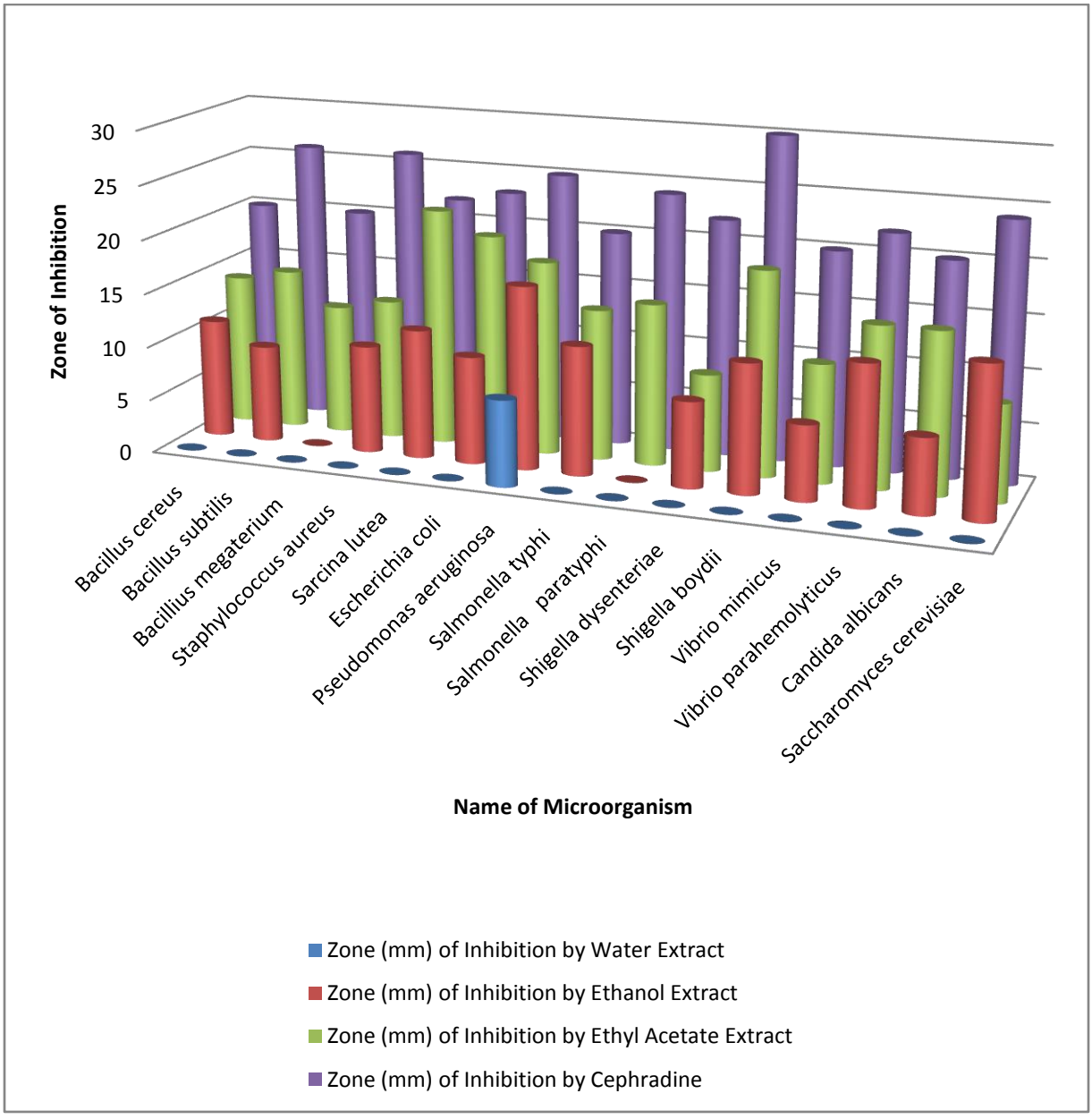


Figure 4.1: Antimicrobial activity of different solvent extracts of at a *Dillenia pentagyna* stem at a concentration of 800µg/disc.

Table 4. 2: Antimicrobial activity of different solvent extracts of at a *Dillenia pentagyna* stem at a concentration of 400µg/disc

Name of Microorganism	Zone (mm) of Inhibition by Water Extract	Zone (mm) of Inhibition by Ethanol Extract	Zone (mm) of Inhibition by Ethyl Acetate Extract	Zone (mm) of Inhibition by Cephradine
Bacillus cereus	0	0	12	20
Bacillus subtilis	0	8	12	26
Bacillius megaterium	0	0	10	20
Staphylococcus aureus	0	7	11	26
Sarcina lutea	0	10	17	22
Escherichia coli	0	8	13	23
Pseudomonas aeruginosa	0	10	14	25
Salmonella typhi	0	10	0	20
Salmonella paratyphi	0	0	13	24
Shigella dysenteriae	0	6	0	22
Shigella boydii	0	8	17	30
Vibrio mimicus	0	0	9	20
Vibrio parahemolyticus	0	10	11	22
Candida albicans	0	0	0	20
Saccharomyces cerevisiae	0	0	0	24

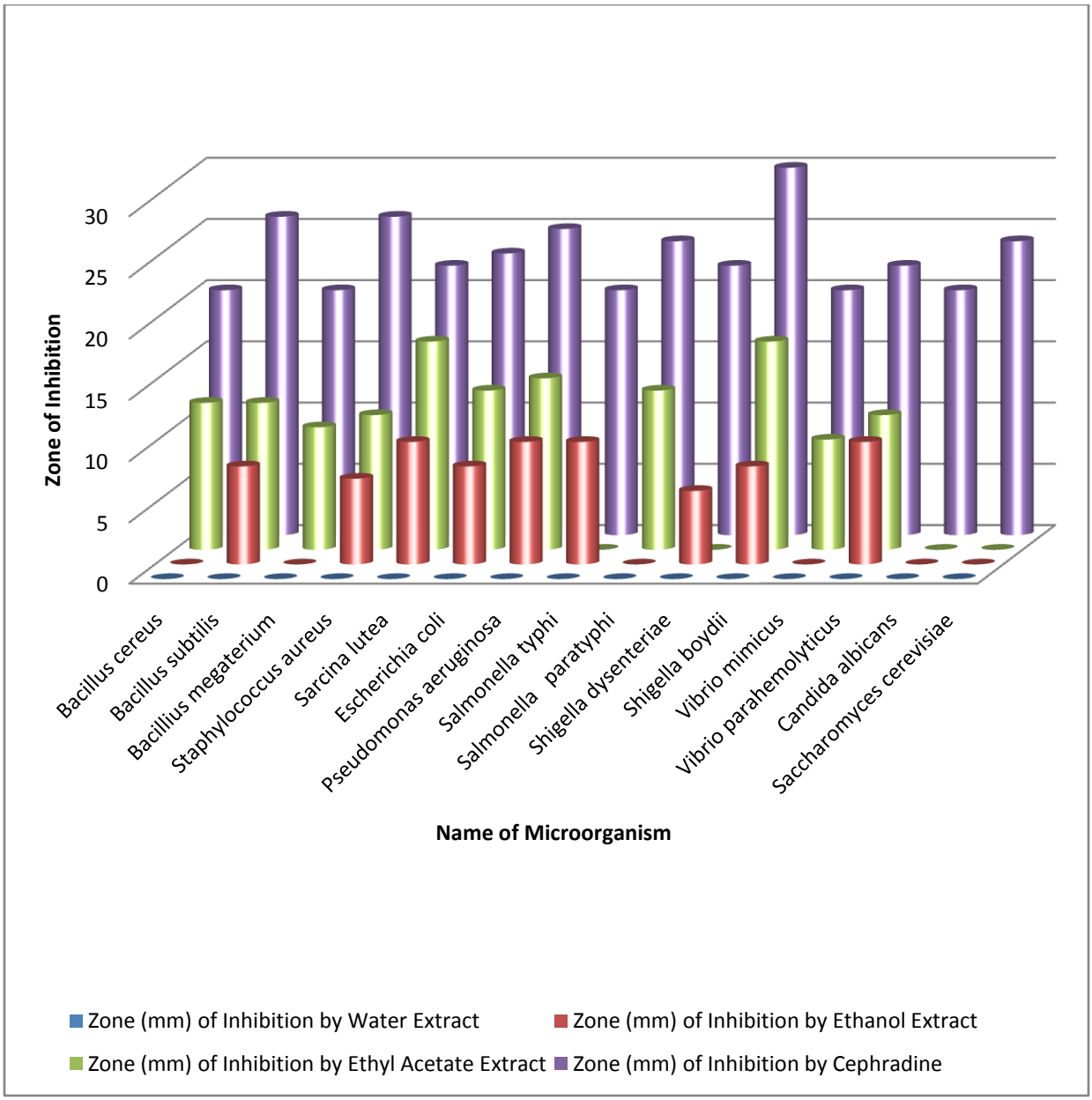


Figure 4.2: Antimicrobial activity of different solvent extracts of at a *Dillenia pentagyna* stem at a concentration of 400µg/disc

Table 4.3: Antimicrobial activity of different solvent extracts of *Dillenia pentagyna* leaf at a concentration of 800µg/disc.

Name of Microorganism	Zone (mm) of Inhibition by Water Extract	Zone (mm) of Inhibition by Ethanol Extract	Zone (mm) of Inhibition by Ethyl Acetate Extract	Zone (mm) of Inhibition by Cephradine
Bacillus cereus	0	11	10	20
Bacillus subtilis	0	12	10	26
Bacillius megaterium	9	12	10	20
Staphylococcus aureus	0	13	11	26
Sarcina lutea	7	15	0	22
Escherichia coli	10	9	10	23
Pseudomonas aeruginosa	0	13	14	25
Salmonella typhi	0	10	0	20
Salmonella paratyphi	6	9	0	24
Shigella dysenteriae	0	0	0	22
Shigella boydii	0	13	10	30
Vibrio mimicus	6	7	0	20
Vibrio parahemolyticus	0	11	10	22
Candida albicans	0	9	0	20
Saccharomyces cerevisiae	0	14	9	24

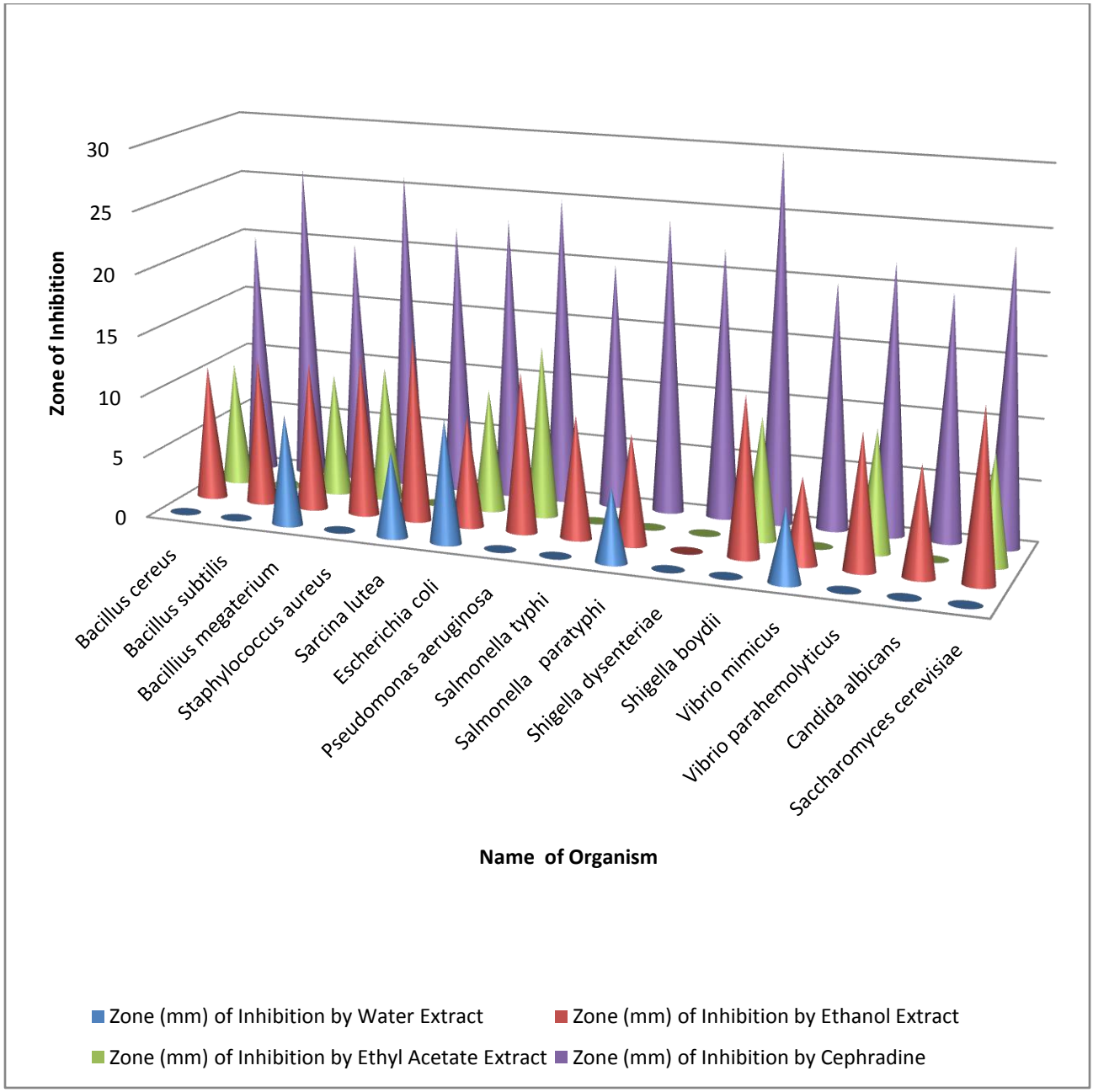


Figure 4.3: Antimicrobial activity of different solvent extracts of *Dillenia pentagyna* leaf at a concentration of 800µg/disc.

Table 4.4: Antimicrobial activity of different solvent extracts of *Dillenia pentagyna* leaf at a concentration of 400µg/disc.

Name of Microorganism	Zone (mm) of Inhibition by Water Extract	Zone (mm) of Inhibition by Ethanol Extract	Zone (mm) of Inhibition by Ethyl Acetate Extract	Zone (mm) of Inhibition by Cephradine
Bacillus cereus	0	10	8	20
Bacillus subtilis	0	7	8	26
Bacillius megaterium	0	0	0	20
Staphylococcus aureus	0	11	7	26
Sarcina lutea	0	12	0	22
Escherichia coli	0	8	9	23
Pseudomonas aeruginosa	8	12	10	25
Salmonella typhi	0	0	0	20
Salmonella paratyphi	0	0	0	24
Shigella dysenteriae	0	0	0	22
Shigella boydii	7	11	7	30
Vibrio mimicus	0	0	10	20
Vibrio parahemolyticus	0	8	0	22
Candida albicans	0	0	0	20
Saccharomyces cerevisiae	0	0	0	24

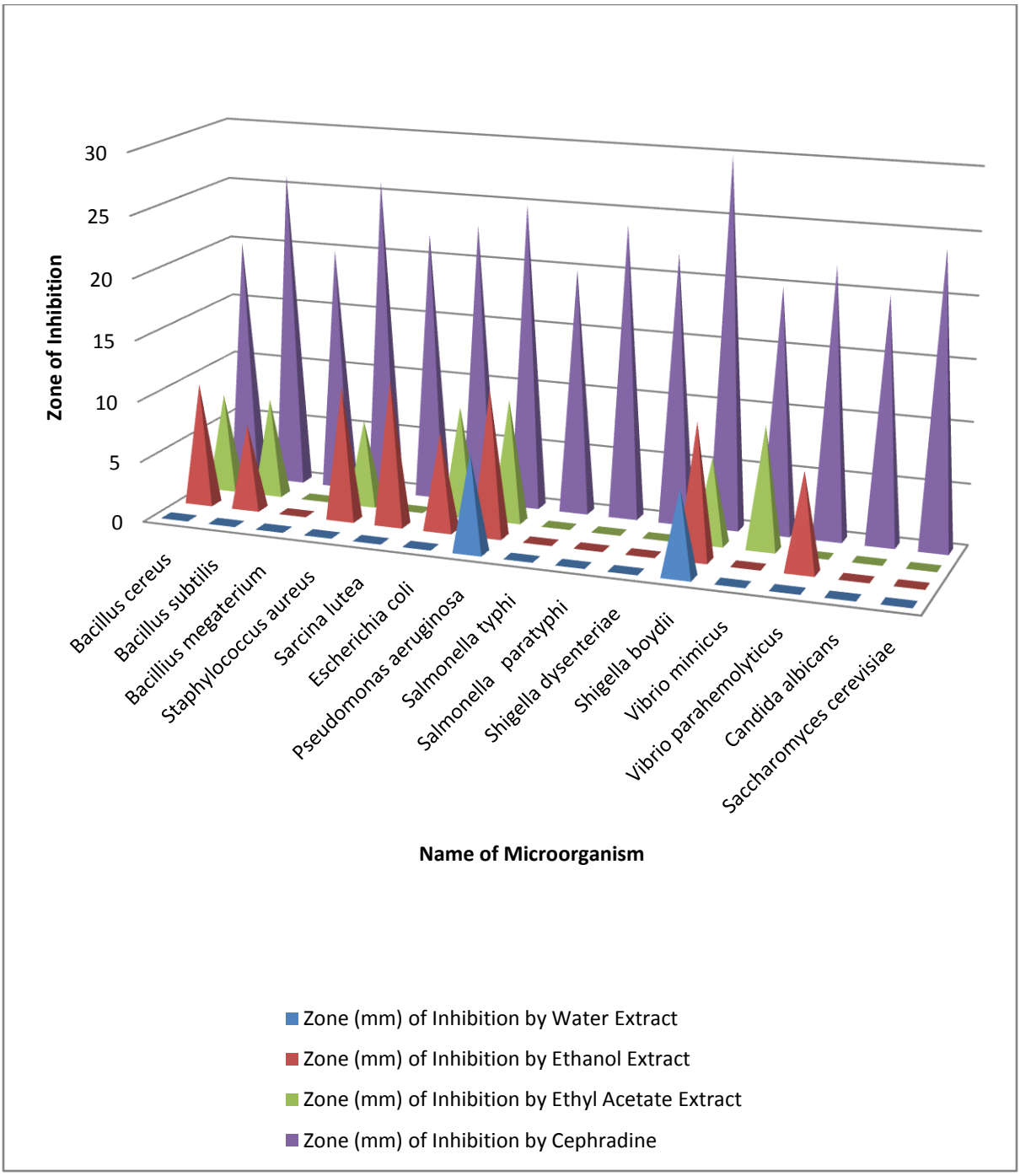


Figure 4.4: Antimicrobial activity of different solvent extracts of *Dillenia pentagyna* leaf at a concentration of 400µg/disc.

4.2 DISCUSSION

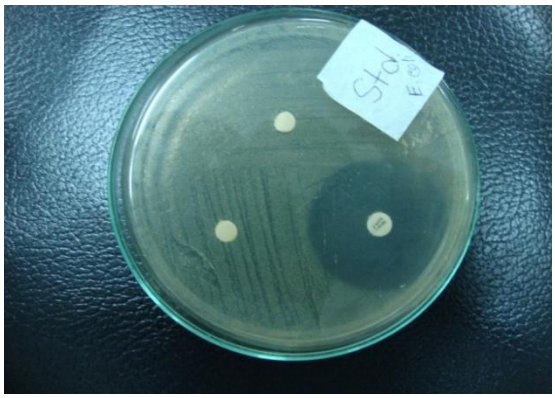
The antimicrobial efficacy of *Dillenia pentagyna* plant extracts (stem and leaf) against bacterial and fungal strains was evaluated by the agar disk diffusion method via determination of the surrounding zones of inhibition (Table 4.1-4.4). The inhibition zone by the plant extracts were ranged from 6mm-18mm**.** The stem extract of *Dillenia pentagyna* was found to be more active than the leaf extract against all the pathogen tested. Among the three solvent extracts (water, ethanol and ethyl acetate), the ethyl acetate extract showed a higher activity than other extracts. This may be due to the solvent extract containing different constituents having antimicrobial activity.

All the gram-positive strains showed sensitivity to ethyl acetate extract of *Dillenia pentagyna stem*, but promising activity found against *Bacillus subtilis and Sarcina lutea* followed by *Bacillus megaterium* and *Staphylococcus aureus*. The ethanol extracts demonstrated sensitivity against *Bacillus subtilis, Bacillus megaterium* and *Staphylococcus aureus.* The water extract did not show any sensitivity.

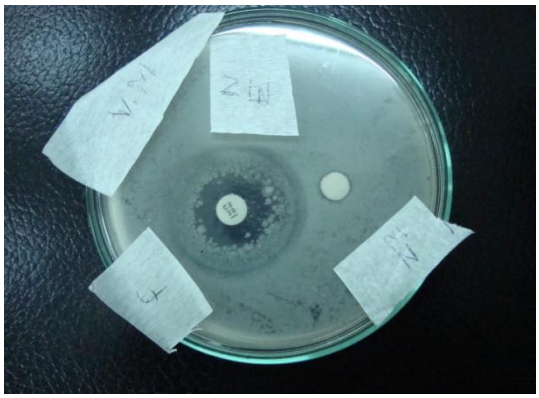
Most of the gram-negative bacterial strains demonstrated more or less sensitivity against ethyl acetate and ethanol extract. But water extract showed less activity to some of the gram-negative strains. Among the gram-negative organisms, *Shigella boydii, Escherichia coli, Salmonella typhi, Salmonella paratyphi, Vibrio parahemolyticus* and *Pseudomonas aeruginosa* exhibited promising sensitivity towards the ethyl acetate extract of stem.

Studies on the antifungal activities showed that ethyl acetate and ethanol extract have shown maximum zone of inhibition against the fungi *Candida albicans* and *Saccharomyces cerevisiae* respectfully. Water extract had no activity.

The results of present study supports that *Dillenia pentagyna* plant extracts containing compounds with antibacterial properties can be used as antibacterial agents in new drugs for the therapy of infectious diseases caused by pathogens and further work may be carried out for phytochemical evaluation.

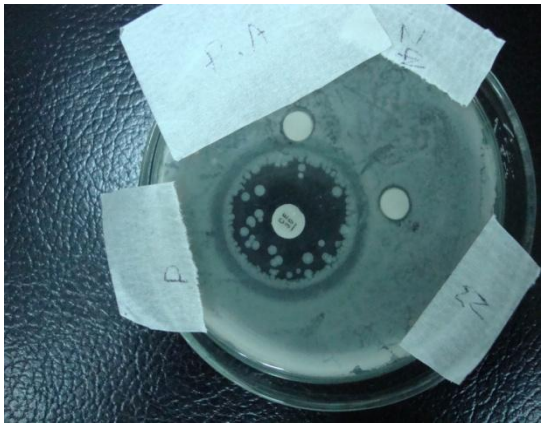


Positive control of *Escherichia coli.*

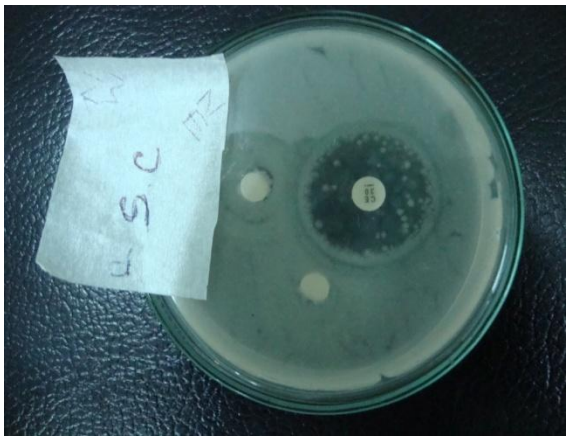


Positive control of *Vibrio mimicus*

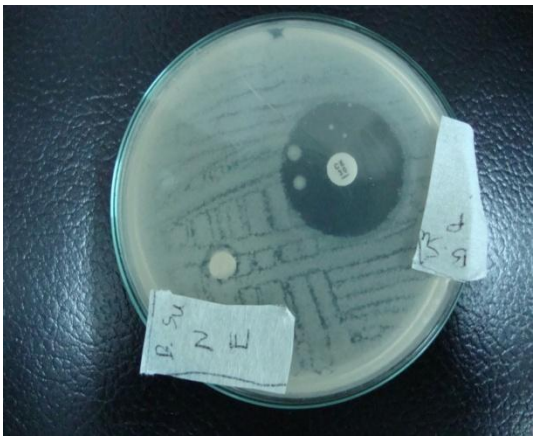
Positive control of *Staphylococcus aureus*



Positive control of *Pseudomonas aeruginosa*

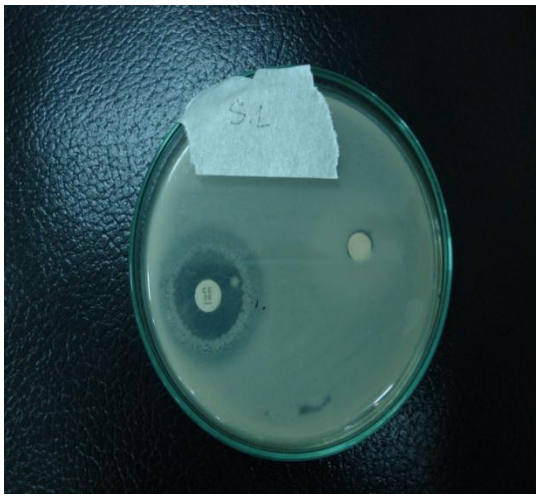


Positive control of *Saccharomyces cerevisiae*

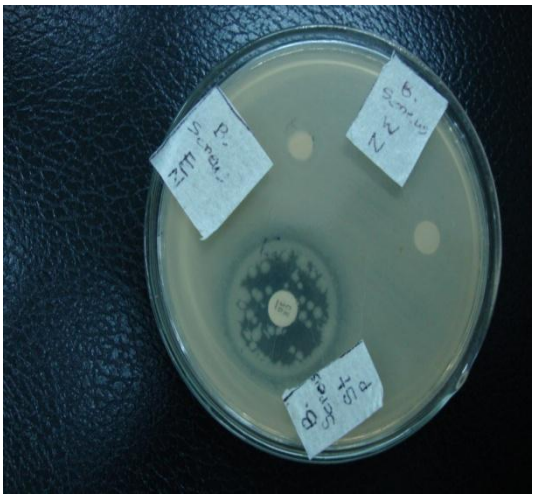


Positive control of *Bacillus subtilis*

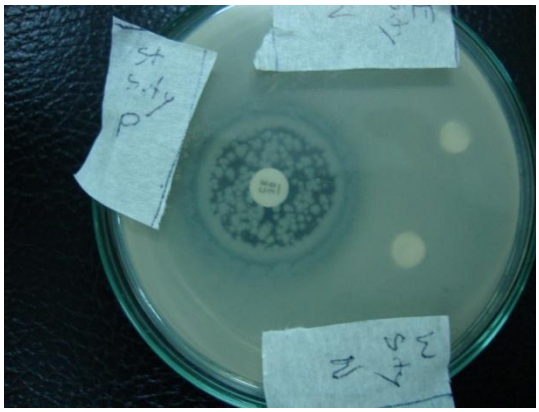
Figure 4.5: Zone of inhibition produced by the antibiotic on the growth of microorganism



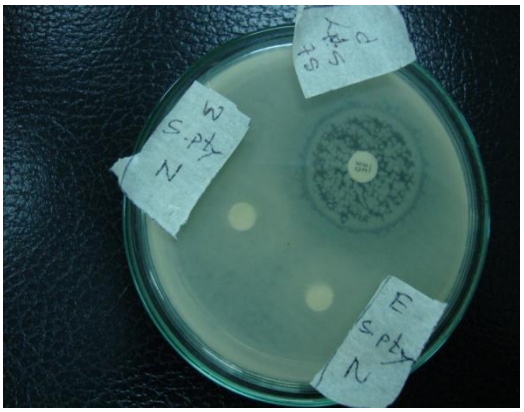
Positive control of *Sarcina lutea*



Positive control of *Bacillus cereus*

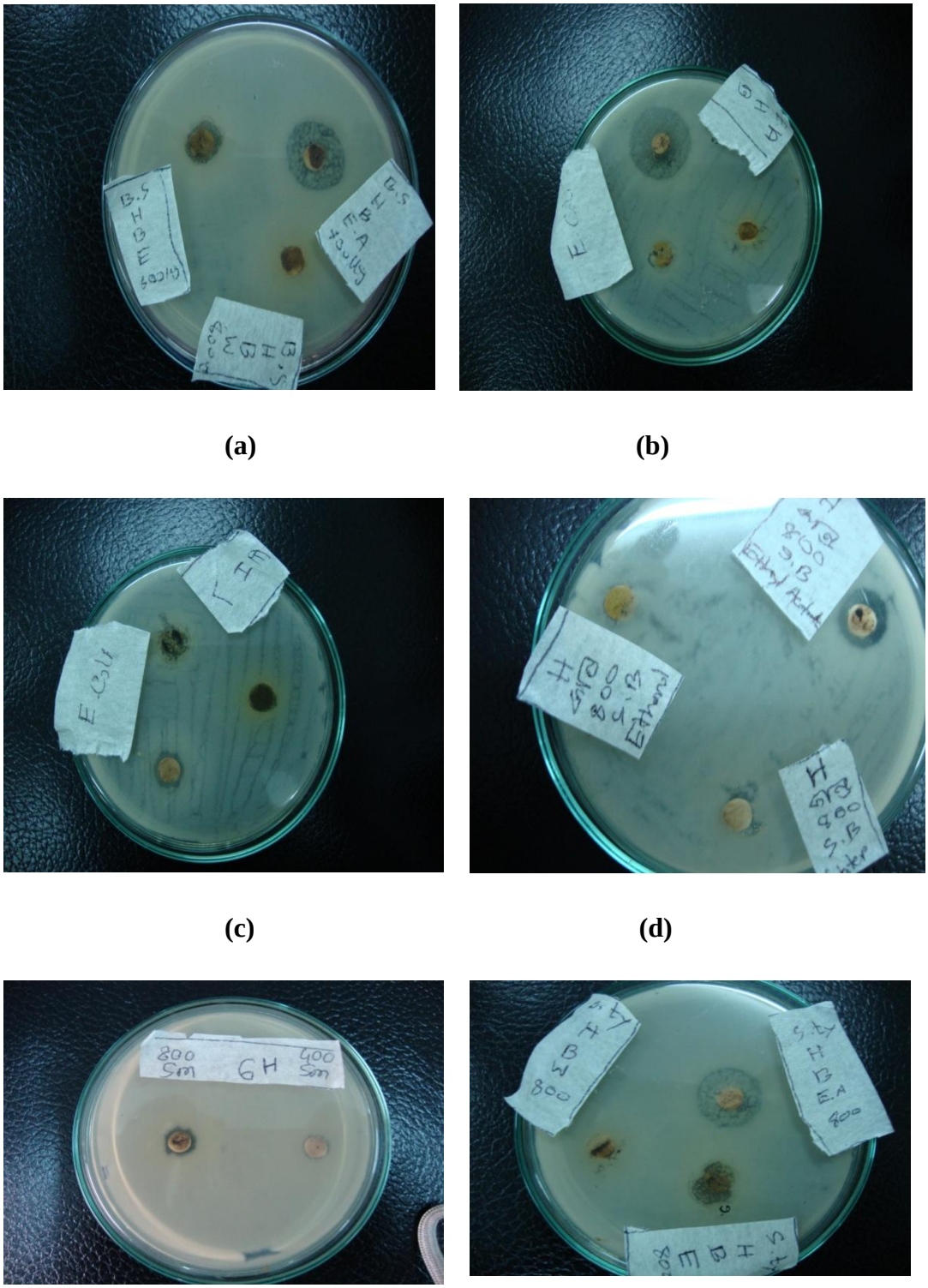


Positive control of *Salmonella typhi*



Positive control of *Salmonella paratyphi*

*Figure 4.*6: Zone of inhibition produced by the antibiotic on the growth of microorganism

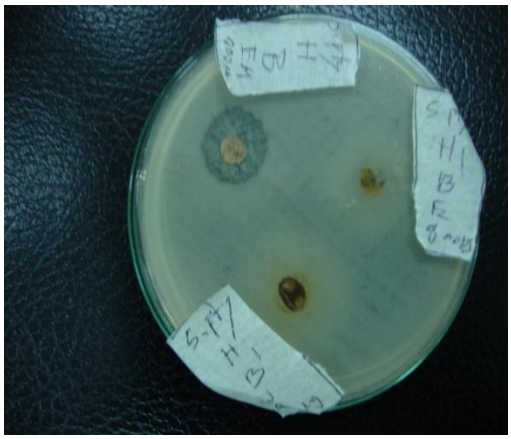


(a) **(b)**

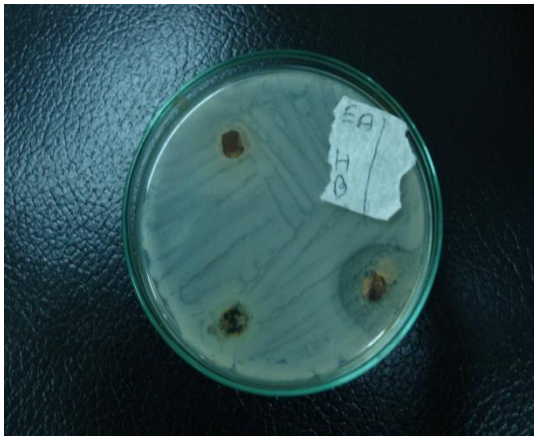
(c) **(d)**

(e) **(f)**

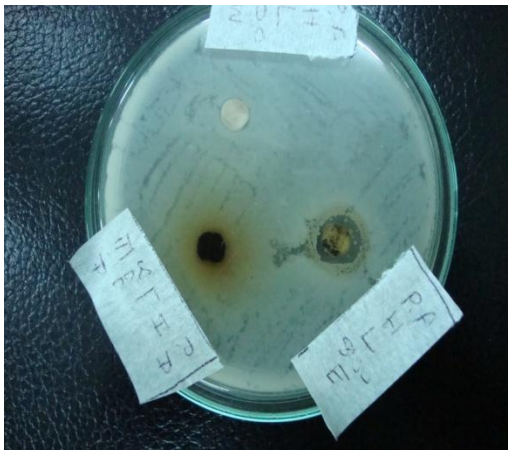
*Figure 4.*7: Zone of inhibition produced by the extracts on the growth of microorganism



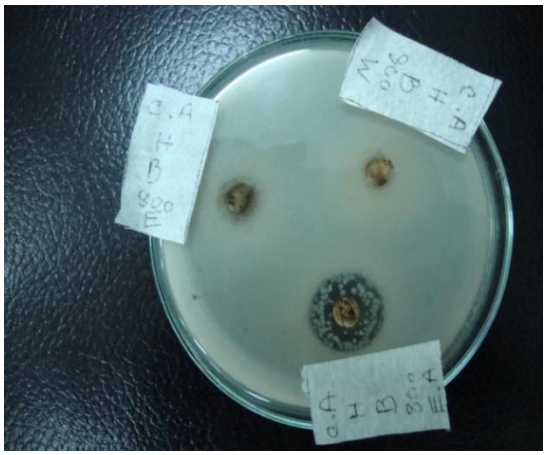
(g)



(h)



(i)



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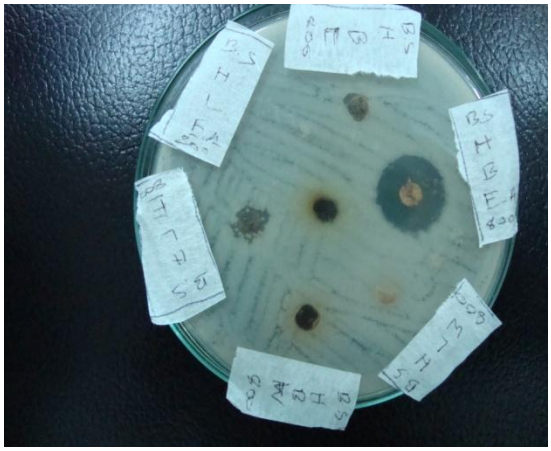
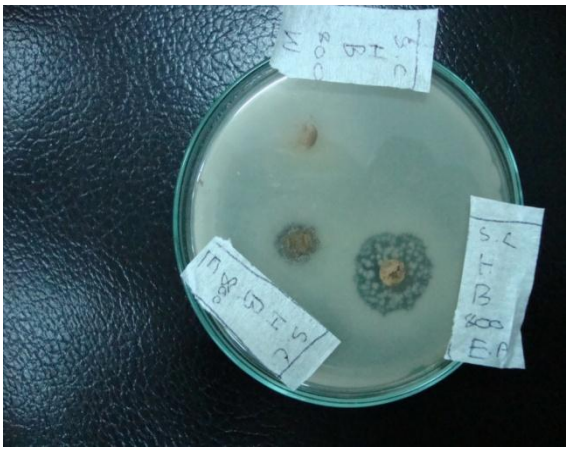


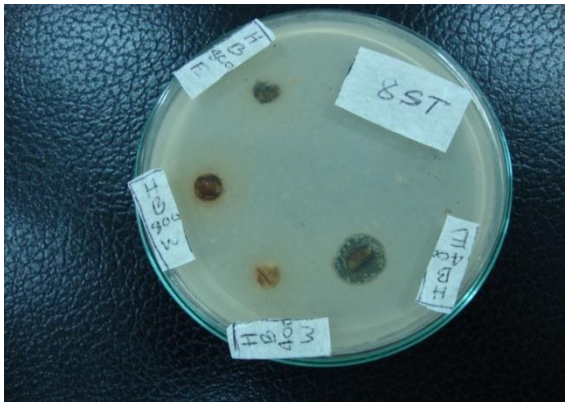
(k)



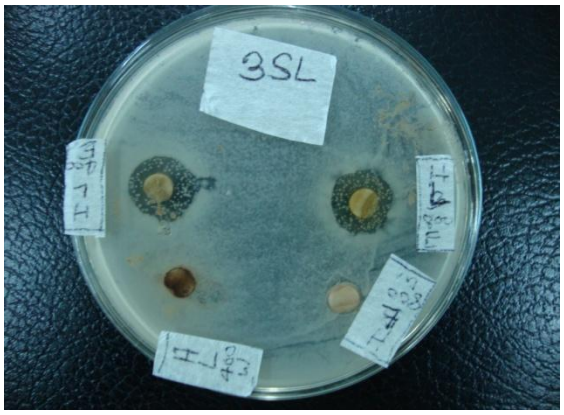
(l)

*Figure 4.*8: Zone of inhibition produced by the extracts on the growth of microorganism

(m)

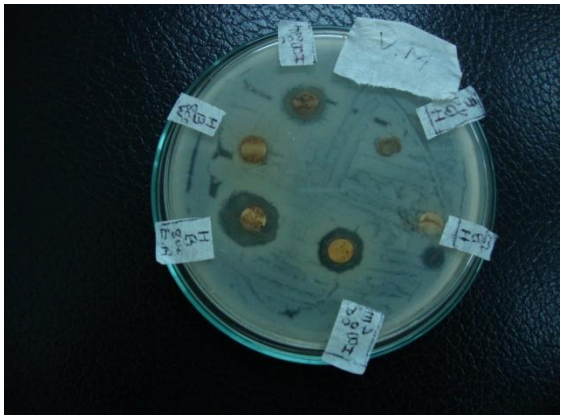


(n)



(o)

(p)



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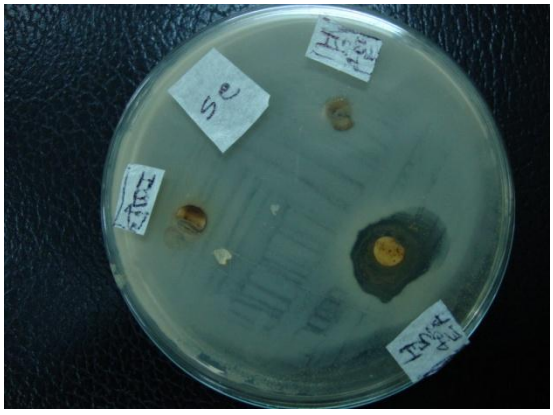
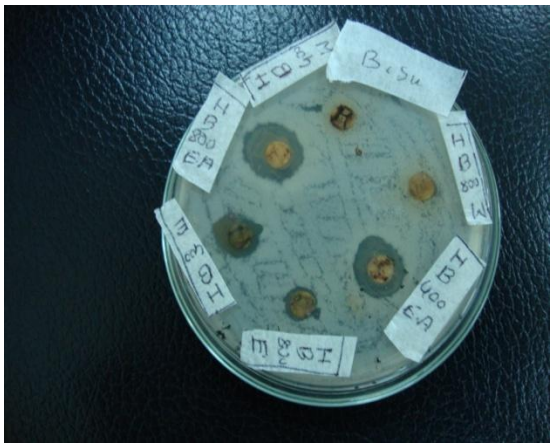
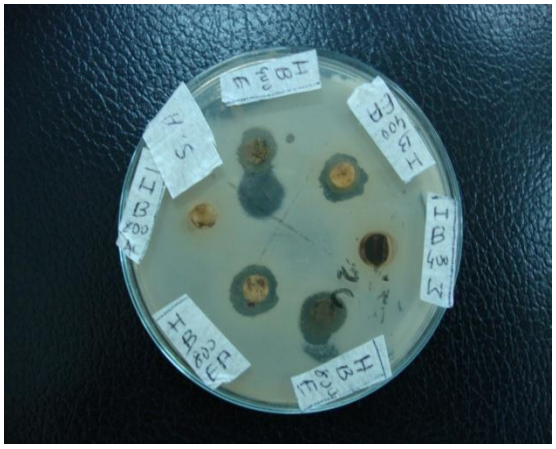


(r)

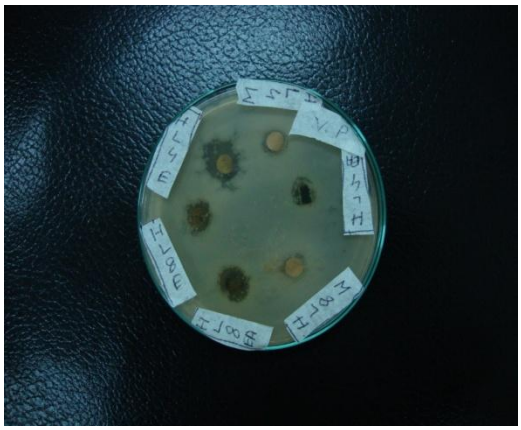
Figure 4.9: Zone of inhibition produced by the extracts on the growth of microorganism



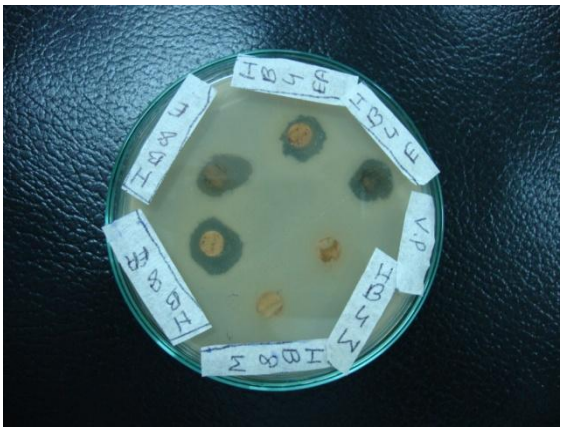
(s)



(t)



(u)



(v)

*Figure 4.*10: Zone of inhibition produced by the extracts on the growth of microorganism

CONCLUSION

The results obtained in this study demonstrated that the medicinal plant, *Dillenia pentagyna* displays *in vitro* antimicrobial activity. The antibacterial activity and antifungal activity seems to confirm the folk therapy of infections and traditional therapeutic claims of this plant. Thus, it has the potential to be used for medicinal purposes specially the ethyl acetate extracts of the species which posses compounds with antimicrobial properties can be used as antimicrobial agents in new drugs for therapy of infectious diseases in human. Isolation, identification and purification of these phytoconstituents and determination of their respective antimicrobial potencies and toxicological evaluation with the view to formulating novel chemotherapeutic agents should be the future direction for investigation.

REFERENCES

1. Sibanda T and Okoh AI. (2007) The challenges of overcoming antibiotic resistance:Plant extracts as potential sources of antimicrobial and resistance modifying agents. African Journal of Biotechnology Vol. 6 (25), pp. 2886-2896.
2. Marjorie MC. (1999) Plant Products as Antimicrobial Agents. Clinical Microbiology Reviews. 12(4): 564–582.
3. G. M. Cragg and D. J. Newman. (2001). Medicinals for the Millenia. Ann NY Acad. Sci. **953**, 3.
4. Dutta U and Sarma G (2008) Koch-Rajbansi's and Their Traditional Phyto-therapy, A Case Study in the Chirang District Of Western Assam. International Journal of Science and Advanced Technology (ISSN 2221-8386), pp59-65.
5. Scientific classification (2012, 25th February). Scientific classification of *Dillenia pentagyna*.
6. Geographical Abundance. (3rd February. 2012). Geographical Abundance *Dillenia pentagyna*.
7. Medicinal uses (2012, 26th February).*Medicinal uses of Dillenia pentagyna*.
8. Gaddam D, shirwaikar A (2004). Ethnomedical value of *Dillenia pentagyna* extract in experimentally induced diarrhoea. *Acta Pharm. 54, 27*–35.
9. Alasbahi, R.H., S. Safiyeva and L.E. Craker, 1999. Antimicrobial activity of some Yemeni medicinal plants. J. Herbs Spices Med. Plants, 6: 75-83.
10. Filipovic, I. and E. Buddecke, 1979. Role of net charge of low-density lipoproteins in high affinity binding and uptake by cultured cell. Biochem. Bioph. Res. Co., 88: 485-490.
11. Imberty, A., C. Gautier, J. Lescar, S. Perez, L. Wyns and R. Loris, 2000. An unusual carbohydrate binding site revealed by the structures of two *Maackia amurensis* lectins complexed with sialic acid-containing oligosaccharides. J. Biol. Chem., 275: 17541-17548.
12. Iwakawa, K., N. Ueda, S. Murao and N. Kobayashi, 1996. Altered carbohydrate composition in colorectal adenomas and carcinomas: Histochemical characterization of

N-acetylgalactosamine, L-fucose and o-acetylated sialic acid. J. Gastroenterol., 31: 24-32.

13. Keiko, M., O. Katsuyuki, I. Mineko, K. Tetsufumi and K. Kensuke *et al*., 2004. Loss of disialyl Lewisa, the ligand for lymphocyte inhibitory receptor sialic acid-binding immunoglobulin-like lectin-7 (siglec-7) associated with increased sialyl Lewisa expression on human colon cancers. Cancer Res., 64: 4498-4505.
14. Warren, L., C.A. Buck and G.P. Tuszynski, 1978. Glycopeptide changes and malignant transformation. A possible role for carbohydrate inmalignant behaviour. Biochim. Biophys. Acta, 516: 97-127.
15. Stringou, E., K. Chondros, J. Kouvaris, S. Kakari and K. Papavassilious, 1992. Serum sialic acid (TSA/LSA) and carcinoembryonic antigen (CEA) levels in cancer patients undergoing radiotherapy. Anticancer Res., 12: 251-255.
16. Serdar, M.A., K. Yilmaz, A. Gunaydin, S. Tapan and K. Caglar *et al*., 2002. Sialic acid content of low-density lipoprotein in hemodialysis patients. Dialysis Transplant, 31: 97-105.
17. Rosangkima, G. and S.B. Prasad, 2004. Antitumour activity of some plants from Meghalaya and Mizoram against murine ascites Dalton's lymphoma. Indian J. Exp. Biol., 42: 981-988.
18. Remani, P., R.A. Nair, T.T. Sreelekha, J. Madhavan, T. Vijayakumar and M.K. Nair, 2000. Altered expression of jack fruit lectin specific glycoconjugates in benign and malignant human colorectum. J. Exp. Clin. Cancer Res., 19: 519-523.
19. Sakagami H, Ikeda M, Unten S, Takeda K, Murayama JI, Hamada A, Kimura K, Komatsu N & Konno K (1987). Antitumor activity of polysaccharide fractions from pine cone extract of *Pinus parviflora Sieb. Et Zucc. Anticancer Res,* **7**,1153-1160.
20. Edeoga HO, Okwu DE & Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants.*African J Biotechnol*, **4**, 685-688.
21. Lalramnghinglova JH (1999). Prospects of ethnomedicinal plants of Mizoram in the new millennium. In: *Symposium on Science and Technology for Mizoram in the 21st Century* (Laltanpuia & RK Lallianthanga, eds.). Aizawl, India, pp. 119-129.

22. Lalramnghinglova JH & Jha LK (1998). Ethnobotanical plants among the hill tribes of Mizoram. In: *Proceeding of International Workshop on Prospects of Medicinal Plants* (PL Gautam, ed.). Indian Society of Plant Genetic Resources, New Delhi, pp. 67-86.
23. Alasbahi RH, Safiyeva S & Craker LE (1999). Antimicrobial activity of some Yemeni medicinal plants. *J Herbs, Spices & Med Plants*, **6**, 75-83.
24. William PJ & Kinghorn AD (2006). Extraction of plant secondary metabolites. In: *Natural Products Isolation* 2nd Edition (DS Satyajit, L Zahid & IG Alexander, eds.). Humana Press, Totowa, New Jersey, USA, pp. 323-352.
25. Huang Paul L, Huang Philip L, Huang P, Huang HI & Huang SL (1992). Developing drugs from traditional medicinal plants. *Chem Industry*, **8**, 290-293.
26. Sharma N, Trikha P, Athar M & Raisuddin S (2000). Inhibitory effect of *Emblica officinalis* on the *in vivo* clastogenicity of benzo[a]pyrene and cyclophosphamide in mice. *Human Exp Toxicol*, **19**, 377-384
27. Chaudhuri T, Sur P, Gomes A, Das SK & Ganguly DK (1998). Effect of tea root extract (TRE) on solid tumors induced by 3-methylcholanthrene in mice. *Phyto Res,* **12**, 62-64.
28. Syiem D, Kharbuli B, Das B, Nongkhlaw DG, Thamar I, Marngar D, Syngai G, Kayang H, Myrboh B, Yobin YSH & Buam DRM (1999). Medicinal plants and herbal medicine: a case study in Meghalaya. In: *Biodiversity, North-east India Perspectives* (B Kharbuli, D Syiem & H Kayang, eds.). Shillong, India, pp. 1-8.
29. Sakagami H, Ikeda M, Unten S, Takeda K, Murayama JI, Hamada A, Kimura K, Komatsu N & Konno K (1987). Antitumor activity of polysaccharide fractions from pine cone extract of *Pinus parviflora Sieb. Et Zucc. Anticancer Res,* **7**, 1153-1160.
30. Edeoga HO, Okwu DE & Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants. *African J Biotechnol*, **4**, 685-688.
31. Harborne JB (1984). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis* 2nd edition. Chapman and Hall London, New York, USA.
32. Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC & Atangbayila TO (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in southwestern Nigeria. *Trop J Pharm Res*, **7**, 1019-1024.