

Running head: ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACT OF  
*Aegle marmelos* STEM BARK

Antibacterial Activity of Methanolic Extract of *Aegle marmelos* Stem Bark

Shabnam Ferdousy (ID-2005-3-70-031)

East West University



In partial fulfillment of the requirements for PHRM 404

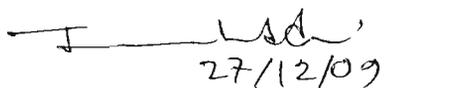
Abu Taiab Md. Jamaluddin

December 27, 2009



## Certificate

This is to certify that the thesis ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACT OF *Aegle Marmelos* STEM BRAK Submitted to the department of Pharmacy, East West University, Dhaka. In partial fulfillment of the requirements for the degree of Bachelor of Pharmacy was carried out by Shabnam Ferdasusy (ID # 2005-3-70-031) under our guidance and supervision and that no part of the thesis has been submitted for any other degree. We further certify that all the sources of information and laboratory facilities availed of in this connection is duly acknowledged.

  
27/12/09

Mr. Abu Taiab Md. Jamaluddin

Supervisor

Lecturer

Department of Pharmacy

East West University, Dhaka

Dr. Chowdhury Faiz Hossain

Professor and Chairperson

Department of Pharmacy

East West University, Dhaka

**DEDICATED TO  
MY BELOVED  
PARENTS**



**ABSTRACT**

*Aegle marmelos* is a medicinal herb belongs to the family Rutaceae. The stem bark of plants is extracted by using methanol. The methanol extracts were screened for the antimicrobial activity. The organisms used were such as *Escherichia coli*, *Salmonella pullorium*, *Staphylococci*, *Bacillus subtilis*. They showed greater inhibitory effect against *Bacillus subtilis*(1.8mm for 15mg/ml and 1.3mm for 20mg/ml) and *Salmonella pullorium* (1.3mm for 15mg/ml and 0.9mm for 20mg/ml). Based on the present investigation results it is concluded that the methanolic extracts of *Aegle marmelos* has potential as antimicrobial agent against different microorganisms and they can be used in the treatment of infectious diseases caused by the resistant microorganisms.

**Keywords:** Antimicrobial activity, *Aegle marmelos*, Clinical pathogens, Disc diffusion technique, Methanol extracts.

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**The Author**

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## INTRODUCTION

*Aegle marmelos* Correa is a sacred tree, dedicated to Lord Shiva. The offering of *bael* leaves is a compulsory ritual of the worship of Lord Shiva in the hills. This importance seems largely due to its medicinal properties. All parts of this tree, viz., root, leaf, trunk, fruit and seed, are used for curing one human ailment or another. *Bael* is a handsome tree, native to northern India, but is found widely throughout the Indian peninsula and in Ceylon, Burma, Thailand and Indo-China (Bailey, 1963). It grows wild throughout the low hills of Himachal Pradesh, ascending up to 1,000 metres. The fruits of the wild trees are, however considerably smaller than those of the cultivated types grown in the plains.

### Scientific classification

Kingdom : Plantae

Order: Sapindales

Family: Rutaceae

Subfamily: Aurantioideae

Tribe: Clauseneae

Genus: *Aegle* (Correa)

Species: *Aegle marmelos*

### Medicinal properties

Watt (1889) reported the unripe dried fruit to be astringent, digestive and stomachic. According to him, they are prescribed to cure diarrhoea and dysentery. The ripe fruit is a good and simple cure for dyspepsia. The roots and the bark of the tree are used in the treatment of fever by making a decoction of them. The leaves are made into a poultice and used in the treatments of ophthalmia. According to Dastur (1962), the rind of the ripe fruit is also sometimes used as a medicine. The roots are sweet, cure the fevers caused by *tridosho*, stop pain in the abdomen, the palpitation of the heart, and allay urinary troubles. They are also useful in the disorders of *vata*, *pitta* and *kapha* (Kirtikar and Basu, 1935). The fruits are very useful in chronic diarrhoea and dysentery, particularly in the case of patients having diarrhoea, alternating with the spells of constipation. Sweet drink (sherbet) prepared from the pulp of fruits produce a soothing effect on the patients who have just recovered from bacillary dysentery, are taken for their mild laxative, tonic and digestive effects. The unripe and half-ripe fruits improve appetite and digestion (Jain, 1968; Jauhari, 1969). The pulp from the unripe fruits is soaked in gingelly oil for a week and this oil is smeared over the body before bathing. This oil is said to be useful in removing the peculiar burning sensation in the soles and also prescribed in cases of hemorrhoids. It has been surmised that the psoralen in the pulp increases tolerance of sunlight and aids in the maintaining of normal skin color. It is employed in the treatment of leucoderma. Marmelosin derived from the pulp is given as a laxative and diuretic. In large doses, it lowers the rate of respiration, depresses heart action and causes sleepiness. A bitter, light-yellow oil extracted from the seeds is given in 1.5 g doses as a purgative. It contains 15.6% palmitic acid, 8.3% stearic acid, 28.7% linoleic and 7.6% linolenic acid. The seed residue contains 70% protein. The bitter, pungent leaf juice,

mixed with honey, is given to allay catarrh and fever. With black pepper added, it is taken to relieve jaundice and constipation accompanied by edema. The leaf decoction is said to alleviate asthma. A hot poultice of the leaves is considered an effective treatment for ophthalmia and various inflammations, also febrile delirium and acute bronchitis. A decoction of the flowers is used as eye lotion and given as an antiemetic. The bark contains tannin and the coumarin, aegelinol; According to Dixit and Dutt (1932), the fruits of *Aegle marmelos* Correa contain a furocoumarin marmalasin, which is responsible for its medicinal properties. The bark contains umbelliferone and other hydroxy coumarins and the alkaloids, fagarine and skimmianine. The bark decoction is administered in cases of malaria. Decoctions of the root are taken to relieve palpitations of the heart, indigestion, and bowel inflammations; also to overcome vomiting. The fruit, roots and leaves have antibiotic activity. The root, leaves and bark are used in treating snakebite. Chemical studies have revealed the following properties in the roots: psoralen, xanthotoxin, *O*-methylscopoletin, scopoletin, tembamide, and skimmin; also decursinol, haplopin and aegelinol, in the root bark.

### Review of Literature

The bael (*Aegle marmelos*) tree grows in tropical and subtropical countries. Various parts of the bael plant are used in Ayurveda and Unani medicine for treatment of a variety of diseases, including treatment of diarrhoea, dysentery and dyspeptic symptoms. In support of this idea, methanolic extracts of unripe fruit from *Aegle marmelos* decreased castor oil-induced diarrhoea in mice, possibly due to the presence of tannin and mucilaginous substances. In addition, patients suffering from diarrhoea-predominant irritable bowel

syndrome showed significantly greater improvement in symptoms when given an indigenous preparation containing *Aegle marmelos* and *Bacopa monniere* compared with placebo. Marmelosin, isolated from the bael plant, has been reported to have anti-helminthic and anti-bacterial activity. No positive effect was seen, however, in a controlled trial examining its efficacy in clinical improvement or bacteriological cure of patients with shigellosis. Further gastroenterological interest in bael comes from the finding that oral administration of luvangetin, a pyranocoumarin isolated from the seeds of *Aegle marmelos*, protected against multiple models of gastric ulceration in rodents. Although the exact mechanism remains unclear, this protection was probably not mediated via prostaglandin pathways. (Ghosh, 2003)

Methanolic extracts of roots of *Aegle marmelos* Corr. And other plants were explored for possible antifilarial effect against *Brugia malayi microfilariae*. It was observed that among the herbal extracts, leaves extract of *Aegle marmelos* Corr. at 100 ng/ml concentration showed complete loss of motility of microfilariae after 48 hr of incubation. Thin layer chromatography of the extracts revealed the presence of coumarin in the leaves of *Aegle marmelos* Corr. (Sahare 2008)

*Aegle marmelos* and other plants were extracted by soxhlet apparatus using petroleum ether, ethanol, chloroform and aqueous as solvent. Among those extract, the petroleum ether was considered as effective one. The extracts were subjected to preliminary phytochemical screening and the three plants with four extracts were tested against three Gram positive bacteria (*B.cereus*, *B.subtilis*, *S. aureus*) and three Gram negative bacteria (*E.coli*, *P.vulgaris*, and *P.aeruginosa*) by disc diffusion method. It also showed inhibitory action against all the six pathogen tested. The zone of inhibition of the extracts was

compared with the standard antibiotics Streptomycin and Spectinomycin. The study suggests that the plant is promising the development of phytomedicine for antimicrobial properties. (Sudharameshwari, 2007)

The protection offered by aqueous extract of *Aegle marmelos* fruit against *Enteropathogenic Escherichia Coli* was due to down regulation of outer membrane protein C , leading to loss of adherence and up regulation of outer membrane protein F , which allowed the entry of  $\beta$ -lactam antibiotics into bacteria. Hence, aqueous extract of *Aegle marmelos*, along with  $\beta$ -lactam antibiotics can be used in treatment of Enteropathogenic *Escherichia coli* infections.

#### **Antibiotic sensitivity test by disc diffusion method:**

Antibiotic sensitivity of both wild and Aqueous extract of *Aegle marmelos* (AEAM) treated Enteropathogenic *Escherichia coli* was qualitatively assessed by the presence or absence of inhibition zone as given in Table. Inhibition zones of Enteropathogenic *Escherichia coli* grown in the presence of AEAM were 3.8, 2.8 and 2.1 for penicillin, ampicillin and vancomycin, respectively. The susceptibility tests were repeated for each antibiotic at least three times.

Table 1: Antibiotic sensitivity test for in absence and presence of AEAM (inhibition zone in mm)

Antibiotics of	Grown in Presence			
AEAM				
Ampicillin(A 10)	0.0	R	3.8	S
Penicillin (P 30)	0.0	R	2.8	S

Norfloxin (N 30)	4.8	S	4.9	S
Vancomycin (Va 30)	0.0	R	2.1	S
Chloramphenicol (C 30)	0.0	R	0.0	R
Novobiocin (Nv 30)	1.0	R	2.4	S
Carbenicillin (Cb 100)	1.2	R	1.2	R

The above table represents inhibitory zones of *Shigella dysenteriae* and *Shigella flexneri* in absence and presence of AEAM. R: Resistance; S: Sensitive (Values in above table represent average of three experiments). (Raja, 2009)

*Aegle marmelos* has been widely used in traditional systems of medicine for a variety of diseases. In the present study, leaves of *Aegle marmelos* were evaluated for its phytochemical contents and antibacterial activity in various extracts of the plant in different solvents of increasing polarity. Antibacterial activity was evaluated by disc diffusion method towards five pathogenic strains of bacteria. The results indicated that the leaves exhibited antibacterial activity especially in methanol extract. Phytochemical analysis indicated that phenol and alkaloids were present in acetone and methanol extracts. Flavanoids were detected only in methanol extract of the plant. Terpenes were not detected in the extracts. Presence of phenols, alkaloids, and flavanoids in methanol extract may be considered as one of the reasons for antibacterial property of leaves *Aegle*

*marmelos*. Minimum inhibitory concentration and minimum bactericidal concentration were also performed for methanol extract towards one of the susceptible organisms, *Serratia marcescens*, it exhibited MIC and MBC values of 200 mg/ml. (Ulahannan, 2008) The petroleum ether, chloroform, methanol and aqueous extracts of *Aegle marmelos* L. Correa. (Fruit) and other plant parts were tested against Enteropathogenic *Escherichia coli* (EPEC). These are the plants traditionally used by rural populace of semi arid regions of India for the treatment of diarrhoea. The ethnopharmacological information on the plants was collected by interviewing the traditional healers, community leaders and rural people of Gujarat State. The agar-well diffusion assay method was used to assess the activities of plant extracts against the test organism. The results obtained show the strong activity of petroleum ether extract of *A. marmelos* and methanol extract of *A. marmelos* and (MIC, 500 µg/ml) followed by petroleum ether extract of *O. basilicum* and chloroform extract of *A. marmelos* and (MIC, 50-100 µg/ml). These preliminary results will be helpful in rationalizing the use of plants based traditional medicines in modern systems of health care. (Patel, 2008)

Fresh leaves of *Aegle marmelos* (L.) Corr., (Rutaceae) were hydrodistilled using a Clevenger apparatus. The resulting essential oils were analysed by gas chromatography/mass spectrometry and antimicrobial activity evaluated against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. The main constituents of *A. marmelos* were sylvestrene (82.49%), sabinene (8.93%) and germacrene D (3.54%). The essential oils exhibited antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli* and *Ps. Aeruginosa*, *A. marmelos* showed the strongest activity against *B. subtilis*. As these plants are already accepted as folk medicines in Thailand and

given the essential oil yield from each plant (i.e. 1.8, 0.5 and 4.3%, respectively), these plants may be suitable for large scale commercial growth and development of a local medicinal essential oil industry. (Kamkaen, 2008)

Antibacterial potential of crude petroleum ether extract obtained from leaf callus tissue of *Aegle marmelos* was evaluated against five bacterial pathogens using agar well diffusion method. Among the tested bacterial strains, inhibitory activity of the callus extract was minimum against *Klebsiella pneumoniae*(4mm) and it was followed by *Proteus vulgaris* (5mm). Maximum inhibitory activity was observed against *Salmonella typhi* (12mm). From this observation it is evident that phytochemical principles which are responsible for the curative (antibacterial) activity of *Aegle marmelos* could have a constant expression pattern in a specialized set of cells even after their rapid division and differentiation into callus form. In vitro micropropagated clones from the parental plant will have an efficient transmission of the antibacterial active constituents. Moreover, this observation may be a baseline for the large scale extraction of antibacterial principles and other curative chemicals through callus culture. (Thangavel, 2008)

Lipid content of 10 oil seeds of different plant families and the antimicrobial activities of these plant seeds that can add a new dimension in the alternative medicinal field of Indian origin. Chemical analysis reveals that the major components of all the seeds were myristic acid, ricinoleic acid, linoleic acid, palmitic acid, lauric acid and oleic acid. The other 4 components i.e., linolenic acid, palmitoleic acid, steric acid and arachidic acid present less than 30% of plant seeds. The primary screening tests for antibacterial and antifungal activities were shown positive for all the compounds. *Escherichia coli* and *Candida albicans* has shown a zone of clearance ranging 11-15 mm whereas

*Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, *Aspergillus niger* and *Penicillium notatum* showed the range between 16-20 mm. It is assumed that oxidative effect could plausibly play an important role in the antimicrobial function of fatty acids. The higher oil content were found to be on *Aegle marmelos* (49%), and can suggest as an agent of conservation in the cosmetic and/or food industries, as an active compound in medical preparations and as a disinfectants.

Table 2: Medical plant of different plant families

Family	Genus and Species	Sample No.
Rutaceae	<i>Aegle marmelos</i>	R5

Table 3: The Oil contents of different oil seeds containing hydroxyl fatty acids

Oil Seeds	Percentage of Oil
<i>Aegle marmelos</i>	49.0

Table 4: Fatty acid composition of different oil seeds represented in (%)

Sam ples	Lau ric	Myri stic	Palm itie	Stea ric	Ole ic	Ricino leic	Linole nic	Linol eic	Arach idie	Palmit oleic
R5	-	4.3	38.8	-	12.	12.5	18.4	13.4	-	-

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Table 5: Screening results for antimicrobial activity of the oil seeds by the agar well diffusion method.

Zone of inhibition (mm)		
Organisms	Control	R5
<i>Escherchia</i>	26	11±1.1

coli		
Pseudomonas	24	20±1.2
aeruginosa		
Salmomella	21	16±1.6
typhi		
Staphyloccus	20	18±0.8
aureus		
Canddida	24	13±0.5
albicans		
Aspergillus	21	19±1.0
niger		
Penicillium	20	17±0.5
notatum		



N.B. diameter of well: 10 mm (Neogi, 2008)

Table 6: Review Table of findings different activity from different parts of *Aegle marmelos*.

Year	Plant parts used	Researcher's Name	Findings
2003	Unripe fruit	Ghosh et al.	Anti-diarrhoeal activity.
2007	Solvent extraction	Sudharameshwari et al.	Antimicrobial properties
2008	Leaves	Sahare et al.	Antifilarial effect

2008	Leaves	Ulahannan et al.	Bactericidal
2008	Petroleum ether extract	Patel et al.	Antibacterial activity
2008	Fresh leaves	Kamkaen et al.	Antibacterial activity
2008	Leaf callus tissue	Thangavel et al.	Antibacterial activity
2008	Oil seed	Neogi et al.	Antibacterial activity and anti fungal activity
2009	Fruit	Raja et al.	Antibacterial activity.

## Materials and methods

### 3.1 Materials

#### 3.1.1 Plant material and extraction

The specimen of *A. marmelos* stem bark was procured from Munshigong, a district of Bangladesh and authenticated from National Herbarium, Mirpur, and Dhaka (Identification No.-BACD Accession No-34358). The stem bark portion was separated from the plant and dried under sun-light for 6 days. After drying, bark part was grinded by using Grinding machine. Total grinded part was about 3kg in weight which was preserved in an air tight and light protected container. Firstly 100mg crude bark powder of *Aegle marmelos* was soaked into 250ml methanol into a beaker. Beaker was covered by aluminum foil and stay for at least 4 days. Every day it was stirred with a clean glass

rod. After 4 days, it was filtered and collected methanolic extract. This liquid part was evaporated to get the solvent free methanolic extract. and concentrated under vacuum using rotary vacuum evaporator and stored desiccated in refrigerator until further use.

**3.1.2 Bacterial strains** Four bacterial strains were employed for the test which include. *Escherichia coli*, *Staphylococci*, *Salmonella pullorium*, *Bacillus subtilis* and were collected from Bangladesh Agricultural University, Mymensingh. The Disc diffusion method was used for determining the antimicrobial activity.

### 3.2 Methods

#### 3.2.1 Diffusion Technique

Antibiotics diffuse from a confine source through the nutrient agar gel and create a concentration gradient. Solutions of known concentration of the test sample are made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper disc containing the test samples of known amount are placed on nutrient agar medium with micropipette uniformly seeded with the test microorganisms. Standard antibiotic (Azithromycin) disc and blank disc (impregnated with solvent) are used as positive and negative control. These plates are kept at low temperature (4 C) for 24 hours to allow maximum diffusion. During this time, dried disc absorb water from the surrounding media and then the test material are dissolved and diffused out of the sample disc. The diffusion occurs according to the physical law that controls the diffusion of molecule through agar gel. As a result there is a gradual change of test materials concentration in the media surrounding the disc.

The plates are then inverted and incubated at 37c for 24 hours for optimum growth of the organisms. The test materials having antibacterial property will inhibit microbial growth

in the media surrounding the discs and thereby yield a clear, distinct area, defined as zone of inhibition. The antimicrobial activity of the test agent is then determined by measuring the diameter of zone of inhibition expressed in millimeter. The experiment is carried out more than once and of the reading is required.

### **3.2.2 Apparatus and Reagent**

Filter paper discs

Sterile cotton

Micropipette

Laminar air flow hood(ESCO<sub>R</sub> Laminar Flow cabinet)

Refrigerator (Samsung)

Petri dishes

Sterile forceps

Screw cap test-tubes

Autoclave(HIRAYAMA,S.I-30208050159)

Nutrient Agar Medium (HIMEDIA,M001.B.No.ZH31)

Inoculating loop

Spirit burner

Nose mask & Hand gloves

Incubator (EHERT)

Sterile tips

Methanol

### **3.2.3 Sterilizing procedure**

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petri dishes and other glassware were sterilized by autoclaving at a temperature of 121°C and a pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

### **3.2.4 Preparation of Culture**

In an aseptic condition under laminar air cabinet, the test organisms were transferred from the collected slants to the Petri dishes containing about 10 ml of melted and sterilized agar medium with the help of a sterilized transfer loop in an aseptic area to have fresh pure cultures. The inoculated strains were then incubated for 24 hours at 37°C for their optimum growth. These fresh cultures were used for the sensitivity test.

### **3.2.5 Preparation of Test Plate**

The test organisms were transferred from the subculture to the test tubes containing isotonic saline with the help of a sterilized transfer loop in an aseptic area. The test tubes were shaken by rotation to get a uniform suspension of the organisms. The bacterial suspension was immediately transferred to the sterilized Petri dishes. The Petri dishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the test organisms in the media.

### **3.2.6 Preparation of Discs**

Three types of discs were used for antimicrobial screening.

**Standard Discs**

These were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of the test sample. In this investigation, Azithromycin was used as the reference standard.

**Blank Discs**

These were used as negative controls which ensure that the residual solvents (left over the discs even after air-drying) and the filter paper were not active themselves.

**3.2.7 Preparation of sample discs with the test sample**

Measured amount of each test sample was dissolved in specific volume of solvent to obtain the desired concentrations in an aseptic condition. Sterilized metrical filter paper discs were taken in a blank Petri dish under the laminar hood. Then discs were soaked with solutions of test samples and dried.

**3.2.8 Preparation and application of test Plant**

The test samples were weighed accurately and calculated amounts of the solvents were added accordingly using micropipette to the dried samples to get desired the concentrations. The test samples were applied to previously sterilized discs using adjustable micropipette under aseptic conditions.

**3.2.9 Diffusion and incubation**

The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria. The plates were then kept in a refrigerator at 4°C for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The

plates were then inverted and kept in an incubator at 37°C for 24 hours.

### 3.3 Determination of Antimicrobial activity

The antimicrobial potency of the test agent is measured by their activity to prevent the growth of the microorganisms surrounding the disc which gives clear zone of inhibition.

After incubation, the antimicrobial activities of the test agent was determined by the measuring the diameter of the zones of inhibition in millimeter with a transparent scale.

## RESULTS

The results of the experiments carried out on the antimicrobial effect on the plant *Aegles marmelos* stem bark with solvent methanol against clinical pathogens Antibacterial activity of methanolic extracts of stem bark *Aegle marmelos* at different concentrations against different clinical pathogens with control was shown (Table).

Table 7. Antibacterial activity against *E.coli* in methanolic extracts of *Aegle marmelos* stem bark.

Concentration of sample	Zone of inhibition produced by sample(in mm)	Zone of inhibition produced by solvent(in mm)	Zone of inhibition produced by the standard disc(in mm)
15mg/ml	No result	0.4	2.2
20mg/ml	1	0.7	1.5

Table 8. Antibacterial activity against *B.subtilis* in methanolic extracts of *Aegle marmelos* stem bark.

Concentration of sample	Zone of inhibition produced by sample(in mm)	Zone of inhibition produced by solvent(in mm)	Zone of inhibition produced by the standard disc(in mm)
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15mg/ml	1.8	No result	2.9
20mg/ml	1.3	No result	3.1

Table 9. Antibacterial activity against *Salmonella pullorium* in methanolic extracts of *Aegle marmelos* stem bark.

Concentration of sample	Zone of inhibition produced by sample (in mm)	Zone of inhibition produced by solvent (in mm)	Zone of inhibition produced by the standard disc (in mm)
15mg/ml	1.3	0.6	No result
20mg/ml	0.9	0.4	No result

Table 10. Antibacterial activity against *Staphylococci* in methanolic extracts of *Aegle marmelos* stem bark.

Concentration of sample	Zone of inhibition produced by sample (in mm)	Zone of inhibition produced by solvent (in mm)	Zone of inhibition produced by the standard disc (in mm)
15mg/ml	No result	No result	4.1
20mg/ml	No result	0.4	3.1

The above table represents inhibitory zones of *Bacillus Subtilis* and *Salmonella pullorium* presence of methanolic extracts of *Aegle marmelos* stem bark is resistant because the zone size of the test strain is smaller than 2mm.



Plate 1. Antibacterial activity of Methanolic stem bark extract of *Aegle marmelos* against *Bacillus Subtilis*

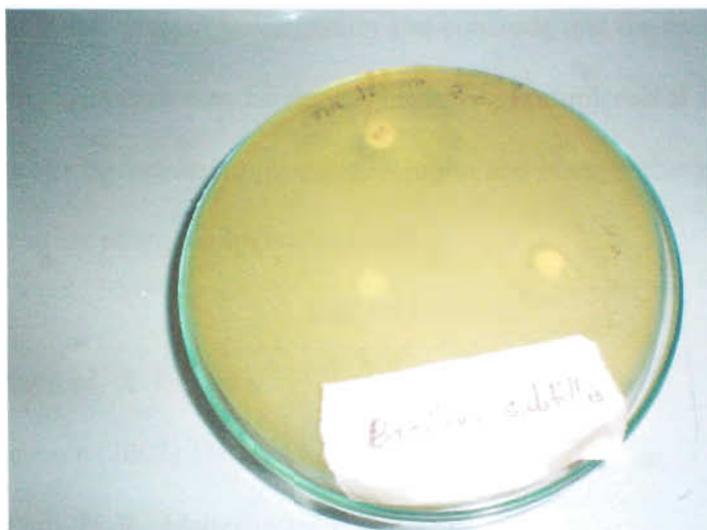


Plate 2. Antibacterial activity of Methanolic extract of *Aegle marmelos* stem bark against *Salmonella pullorum*

## DISCUSSION

The stem bark methanol extract was found to be resistant against *Bacillus subtilis* and *Salmonella pullorium* on the basis of zone size interpretation. Each zone size is interpreted as follows:

1. Sensitive: The zone size is equal to, larger than or not more than 3 mm smaller than the control.
2. Intermediate: The zone size of the test strain is at least 2 mm, but also at least 3 mm smaller than that of the control strain.
3. Resistant: The zone size of the test strain is smaller than 2 mm.

But earlier result as antibacterial activity of *Amry* card power (formulation) consists of *Aegle marmelos* against *E. coli*, *Staphylococcus* and *Streptococcus*. On the basis of the result obtained in this present investigation and conclude that the methanol extracts of *Aegle marmelos* stem bark may be significant in vitro antimicrobial activity and the most active extracts can be farther subjected to isolation and identify therapeutic antimicrobials and undergo further pharmacological evaluation.

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