Local Varieties of *Allium Sativum* Has More Effective Antimicrobial Property Compared to Imported Variety

A DISSERTATION SUBMITTED TO BRAC UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE IN MICROBIOLOGY

Submitted by:
AakibParvez
Student ID: 13326009
July, 2018

Microbiology Program
Department of Mathematics and Natural Sciences
BRAC University
Dhaka

Declaration

I, AakibParvez declare that this thesis and the work entitled "**Local Varieties of *Allium Sativum* Has More Effective Antimicrobial Property Compared to Imported Variety**" submitted to the Department of Mathematics and Natural Sciences (MNS), BRAC University in partial fulfillment of the requirements for the degree of Bachelor of Science in Microbiology is a record of work carried out by me under the supervision of my supervisor, **Mahbubul Haque Siddiqee**, Lecturer, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University, Dhaka.

It is further declared that this thesis has been composed solely by me and it has not been submitted, in whole or in part, in any previous institution for a degree or diploma. All explanations that have been adopted literally or analogously are marked as such.

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Aakib Paevez

Candidate

_____

Mahbubul Haque Siddiqee

Lecturer

Microbiology Program
Department of Mathematics and Natural Sciences
BRAC University, Dhaka
Supervisors

Acknowledgement

Firstly, I would like to give thanks to Almighty Allah because for His blessings and the strength, He gave me during my research work and the opportunity to finish this research against all odds.

I acknowledge my esteem to Professor A F M Yusuf Haider, Chairperson of MNS Department, late Professor A. A. Ziauddin Ahmad, Ex- Chairperson of MNS Department and Professor Dr. Mahboob Hossain, Coordinator of Microbiology Program of MNS Department of BRAC University for allowing me and encouraging me to complete my undergraduate thesis.

My regards, gratitude, indebtedness and appreciation goes to my respected supervisor **Mahbubul Haque Siddiqee**, Lecturer, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University for his constant supervision, constructive criticism, expert guidance, enthusiastic encouragement to pursue new ideas and a good sense of humor and never ending inspiration throughout the entire period of my research work.

I also express my heartiest gratitude to **Fahareen-Binta-Mosharraf**, Lecturer, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University who were abundantly helpful and offered invaluable assistance, support and guidance. Without her supervision, cooperation and valuable suggestions it would be very difficult to complete the research.

I would also like to express my sincerest gratitude to the respective Lab officers Shamim Akhter Chowdhury and Asma Binte Afzal, Teaching assistants Nahreen Mirza and Salman Khan for their suggestions, relentless support, continuous assistance and endless encouragement during my work.

My sincere appreciation is extended to staffs of the department and my friend Anzinur Nahar Samantha, who helped me even beyond their duty period to continue my research work.

Last but not the least; I would like to give my gratitude to my mother and sister for moral support and inspiration which gave me the strength to complete my research work.

Abstract

Natural antibiotics or commonly known as herbal plants are used against various small diseases throughout the history of mankind. Now we use synthetic antibiotics. However, these antibiotics are very much ineffective against various microorganisms because of the antibiotic resistance. In current work we used Bangladeshi and hybrid garlic against *Vibrio cholerae*, *Staphylococcus aureus*, *Bacillus cereus* and *Streptococcus pyogenes.* The garlic samples were dried and ethanolic and methanolic extract were prepared following a previously published protocol. The extracts were diluted by Dimethyl sulfoxide and Distilled water at 75%, 50% and 25% for conducting agar diffusion tests. The prepared extracts were tested on microorganisms by using minimal bactericidal concentration of garlic extract. Further, agar well diffusion test were performed in order to determine the zone of inhibition. For determine the qualitative analysis of the phytochemical properties of Bangladeshi and hybrid garlic, we did basic phytochemical screening tests. Bangladeshi variety of garlic showed MBC of 62.5%, 67.5%, 52.5%, and 65% against *Vibrio cholerae*, *Staphylococcus aureus*, *Bacillus cereus* and *Streptococcus pyogenes respectively* where as MBC of 70%, 75%, 70%, and 70% was found for the hybrid variety against the same organisms. The average diameters of inhibition-zone were 11mm, 12.5mm, 21.5mm and 18.65mm against *Vibrio cholerae*, *Staphylococcus aureus*, *Bacillus cereus* and *Streptococcus pyogenes* respectively while the same for hybrid variety were 8mm, 6mm, 20.67mm, and 7mm These results suggest that the local variety might be more effective against the bacteria tested in this study. The phytochemical test result shows that Bangladeshi Garlic contains protiens, tannins and phenols, alkaloids and tritenpenoids on the other hand hybrid garlic has proteins and alkaloids but tritenpenoids was absent. Overall, the result shows that Bangladeshi garlic might be more effective against microorganisms and it can act as a stepping stone to use garlic for therapeutic purposes.

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List of abbreviations

DH_2O: Distilled water

DMSO: Dimethyl sulfoxide

MIC: Minimal inhibitory concentration

MBC: Minimal bacteriocidal concentration

MHA: Muller hinton agar

NB: Nutrient agar

HPLC: High performance liquid chromatography

Introduction

1.1 Background

In south Asian country the use and development of natural product as medicine started thousands of years ago. It helped them to increase the knowledge about the interrelation between biological properties and chemical structures and to understand the close relationship between animal and plants. (Viegas C, 2006) That is why medical plants are important substance to study with the verification of the medical science. These medical plants can be used for composite source of new anti-microbial agents. The microbial infections are the major cause of morbidity and mortality of developed and developing country, although a number of antimicrobial agents are available for the treatment and management of infectious diseases. In addition, misuse of the antibiotics which can lead to the development of antibiotic resistance is also a major health concern (Al-Bari MAA, 2007). That is why we need to exploit the various medical plants and natural compounds in order to fight the microbial infections which are resistant to stereotypical antibiotics.

Use of different plant parts as medicine has been a traditional practice all over the world. Herbalism is now the alternative solution beside antibiotics to fight against MDR organisms. Plant naturally develops antimicrobial properties and so plant extracts are used for medicinal purpose (Akram, 2007).

1.2 Background information on garlic

Garlic (*Allium sativum*) has a long history as a treatment for cold, cough and asthma, and is reported to strengthen the immune system (C, 2001). Moreover, garlic is widely used to treat alzheimer's disease, cancer, cardiovascular disease including atherosclerosis, strokes, hypertension, thrombosis and hyperlipidemias, infections, enhance children's conditions and dermatologic applications, as well as reduce stress (Bongiorno PB, 2008). Generally, garlic has long been known to have antibacterial, antifungal, anticancer and antiviral properties (Ekweney UN, 2005). The main antimicrobial constituent of garlic has been identified as the oxygenated sulfur compound, thio-2-propene-1-sulfinic acid S-allyl ester, which is referred to as allicin (Cavallito CJ, 1944).

Domain	Eukarya
Kingdom	Plantae
Clade	Angiosperms
Clade	Monocots
Order	Asparagales
Family	Amaryllidaceae
Sub family	Allioideae
Genus	*Allium*
Species	*A.sativum*

Table 1.1- Scientific classification of garlic (Plant Database, 2015)

Figure 1.1: Garlic and garlic bulb

1.3 Objective of the study

- Ethanolic and methanlic extraction of powdered garlic
- Find the minimal inhibitory concentration(MIC) of the garlic extract
- Find the Minimal bacteriocidal concentration(MBC) of the garlic extract
- Use the extract to check the antimicrobial activity by agar diffusion method against sensitive strains
- Find the phytochemicals properties of garlic.

Methodology

2.1 Preparation for plant material

the fresh from of garlic is collected from 3 different district of Bangladesh and 2 sample of Indian garlic were collected from Dhaka Karwanbazar, Sherpur and Pabna. They were identified in Botanical survey of Bangladesh. The fresh forms of garlic cloves were cut into small pieces and air-dried and made into powdered forms using an electric blender of microbiology lab of BRAC University.

2.2 Extraction of Plant Material

The extraction method used know as Fatope et al (Fatope AO, 1993), according to this method I dissolved 20g of powdered garlic with 400ml of ethanol and methanol respectively in conical flasks at room temperature (thus achieving 1:20 ratio) the conical flask were covered with aluminum foil paper, shaken and left in a shaker incubator at 37°C for 2 weeks at regular shaking. After 2 weeks the suspensions were filtered by using Whatman filter papers and then the garlic suspension was concentrated in Rotary-evaporating machine at 40°C. The extract turned in semi-solid which labeled accordingly and stored in the refrigerator for further analysis.

Figure 1.2: Garlic extract

2.3 Extract preparation for antimicrobial test

The ethanolic and methanolic crude of garlic sample we dissolved in distilled water (DH_2O) and Dimethyl sulphoxide (DMSO) to create 25%, 50% and 75% diluted solution. The garlic extract was diluted in sterile distilled water and DMSO for achieving the concentration of 25%, 50% and 75%. These diluted extract were prepared foe determination of antimicrobial activity, minimal inhibitory concentration and minimal bactericidal concentration.

2.4 Test pathogenic bacteria

For these study four strains of bacteria was selected from the microbiology laboratory of BRAC University. These bacteria are *Vibrio cholera, Staphylococcus aer*ues,*Bacillus cerues* and *Streptococcus pyogenes*

2.5 Anti Microbial activity of Garlic extract

The anti-microbial activity of garlic extract was tested by three methods.

1. Minimum inhibitory concentration (MIC)
2. Minimum bactericidal concentration and (MBC)
3. Agar diffusion methods

2.6 Minimum inhibitory concentration

Minimal inhibitory concentration or MIC of garlic extract is the minimum concentration by which the bacterial growth is inhibited

2.6.1 Preparation of inoculums

The inoculums were prepared by taking a 16-24 hours' culture of the organism on nutrient agar plate. One of the colonies was picked with a sterile loop and mixed with sterile normal saline. The bacterial suspension was prepared using a vortex machine. The optical density of the suspension was measured at 600 nm wavelength using a Spectrophotometer. The absorbance of light by the bacterial suspension was recorded and adjusted to 0.5 McFarland standards. The absorbance of 0.5 McFarland standards would be between 0.8- 1.0 at 600 nm wavelength of light beam to travel a distance of 1 cm pathway. If the 0.5 McFarland Standard was set by the absorbance between the above mentioned ranges, then the bacterial suspension in the cuvette may contained around 1.5 x 108 CFU.

2.6.2 Preparation of extract and broth medium

Concentrated garlic extract prepared for MIC by diluting with DMSO. These stock extract were serial diluted in the range from 100 to 1000 µg/ml by using DMSO. Every test-tube were labeled from number 1 to 12. 1ml of NB was added to every test-tube. Test-tube number 1 has 1ml of NB and 1ml of stock 100% extract thus it is the positive control on the other hand test tube number 12 does not have any extract in it thus it is the negative control.

After adding the NB then 500µl of selected bacterial culture was added to every test-tube except for test-tube number 1 then the test tubes were mixed using vortex machine. Then the test tubes were incubated for 24 hours at 37^{o} C.

2.7 Minimum bactericidal concentration

Minimum bactericidal concentration (MBC) was required to find out the efficiency of any antimicrobial agent. It indicates the minimum concentration of the antimicrobial agent required to kill the bacteria. It was determined after determining the MIC of the antimicrobial by setting up a series of dilution of antimicrobial agent followed by inoculation of bacteria as in determining MIC. After the overnight incubation at 37ºC the cultures were sub-cultured by spreading from the solution on MHA by a sterile glass spreader for bacterial growth. The speared MHA were incubated overnight again in 37°C in an incubator. Next day, the bacterial growths of respective solutions were going to give the idea about the MBC of the extract.

2.8 Agar diffusion method

Agar diffusion method is a basic test for determine the capacity of an anti-microbial agent to inhibit bacterial growth. There are two types of agar diffusion method, one is disc diffusion and another is well diffusion

In disc diffusion method the sterile discs were prepared with Whatman filter paper then the extracts were soaked to the discs and dried. The dried discs were placed on microbe matted MHA plates. The anti-microbial agent from the disk gets diffused in the agar and creates a clear zone around disk. On the other hand, in well diffusion method, a hole is punched in the agar plate using a cork borer. A definite volume of the anti-microbial agent is added. To that hole the anti-microbial agent diffuses to the agar and creates a clear zone around the hole. If the organism is resistant to the anti-microbial agent no clear zone appears.

For well diffusion method MHA plates were used for bacterial growth. Not more than 24 hours cultured were used for the well diffusion method. The selected bacterial culture was inoculated into test-tube containing NB and incubated for 2-3 hours. After the short incubation period the enriched bacterial cultures were lawned into MHA by using a sterile cotton swab. After that holes were punched on the inoculated agar plate with sterile 5mm cork borer. In every single punched holes 50µl extract and control were placed and after that the plates were incubated overnight at 37^{o}C.

2.9 Quality control

Quality control is a very important step in any experiment. Quality control helps to compare the results obtained with a positive response and a negative response to ensure there is no positive or negative impact on the result.

2.10 Quality control for agar diffusion test

This control was set up to check whether any other components, for example, the solvent, DMSO have had any effect on the bacterial strains or not. The amount of DMSO used to dissolve the garlic extract and total volume of the dissolved extract were recorded. The same volume of DMSO was taken in a test- tube and total volume of DMSO was adjusted with 0.2 µm filtered and autoclaved deionized water. Control for the extract was diluted in the same way as the extract itself.

2.11 Quality control for minimum inhibitory concentration of the Garlic extract

The quality control for the minimum inhibitory concentration of the garlic extract was setup by keeping a negative control and a positive control for the test organisms. From the series of dilutions of the garlic extract, one concentration was set to 0 mg/ml, which means no garlic extract, was added to those set vials. This concentration was used as negative control. In one test-tube no bacterial colonies were inoculated with the extract, which serves as the positive control for the test.

2.12 Phytochemical screening

Phytochemistry is the branch of chemistry, which deals with the chemical nature of the plant or plant products (chemistry of natural products). Plants contain many chemical properties, which are therapeutically active or inactive. A spectrum of natural compounds like triterpenoids, alkaloids, glycosides, tannins, flavonoids, essential oils and other similar secondary metabolites which exert physiological activities are synthesized in the plant, in addition to the carbohydrates, proteins and lipids utilized by man as food material. (Kokate C K, 2002)

Experimental Methods

The selected plants extracts were taken for qualitative chemical investigation to test the presence of various phytochemicals in garlic.

2.11.1 Test for proteins

• **Biuret's Test:** To 3 ml of extract 1 ml of 4% w/v sodium hydroxide and 1ml of 1% w/v copper sulphate were added. The change in color of the solution to violet or pink indicates the presence of proteins.

2.11.2 Test for Tannins and phenols

• **Lead acetate Test**: To 3ml of extract, 3 ml of lead acetate solution was added. The occurrence of white precipitates indicates the presence of tannins and phenols.

2.11.3 Test for Glycosides

• **Keller-killiani test:** To the test tubes containing 2 ml of extract 1 ml of glacial acetic acid, 3 drops 5% W/V ferric chloride and concentrated sulphuric acid were added and observed, disappearance of reddish brown color at the junction of two layers and bluish green in upper layer indicates the presence of cardiac glycosides.

2.11.4 Test for saponins

• **Foam Test**: The extract (2g) was shaken vigorously with 20 ml of water and observed for persistent foam, which indicates the presence of saponins.

2.11.5 Test for flavonoids

• **Shinoda Test**: To the dry extract (2g), 5 ml of ethanol (95% v/v), 5 drops of hydrochloric acid and 0.5g of magnesium turnings were added. Appearance of pinkcolor indicates the presence of flavonoids.

2.11.8 Test for Triterpenoids

• **Salkowaski Test:** To 2 ml of extract 5 drops of concentrated sulphuric acid was added, shaken and allowed to stand. Appearance of greenish blue color indicates the presence of triterpenoids.

2.11.7 Test for Alkaloids

To the 10 g of dry extracts, 20 ml of dilute hydrochloric acid was added, shaken well and filtered. The following tests were performed using the filtrate.

• **Wagner's Test**: To 3 ml of filtrate, 1ml of Wagner's reagent (iodine in potassium iodide) was added. The appearance of reddish brown precipitate indicates the presence of alkaloids.

• **Hager's Test**: To 3 ml of filtrate, 1ml of Hager's reagent (saturated picric acid solution) was added. The appearance of yellow precipitate indicates the presence of alkaloids.

• **Dragendroff's Test**: To 3ml of the filtrate, 1ml of Dragendroff's reagent (potassium bismuth iodide) was added. The appearance of brick red precipitate indicates the presence of alkaloids.

Results

3.1 Result of Minimal Inhibitory Concentration of Garlic extract on selected bacteria

After the 24-hour incubation period the vial containing Garlic extract and NB and bacterial suspension were observed by eye however the extracts were mixed with the NB that is why in order to determine the result of MIC the solutions were spread on to MHA thus conducting Minimal bactericidal concentration(MBC).

3.2 Result of Minimal Bactericidal Concentration of Garlic extract on selected bacteria

Minimal bactericidal concentration of garlic extract was determining by culturing the solutions of NB, extract and bacterial solution on to MHA and incubated overnight to see the growth of the bacteria. The results are given below.

Extract: Ethanol Garlic BD Type-1

		Concentration of garlic extract										
Organisms	Positive control	100%	90%	80%	70%	60%	50%	40%	30%	20%	10%	Negative control
Vibrio cholerae	+	+	+	+	+	+	-	-	-	-	-	-
Staphylococcus aerues	+	+	+	+	+	-	-	-	-	-	-	-
Bacillus cereus	+	+	+	+	+	+	+	-	-	-	-	-
Streptococcus pyogenes	+	+	+	+	+	-	-	-	-	-	-	-

Table3.1: MIC result of ethanol garlic BD Type-1

Extract: Ethanol garlic BD Type-2

		Concentration of garlic extract										
Organisms	Positive control	100%	90%	80%	70%	60%	50%	40%	30%	20%	10%	Negative control
Vibrio cholerae	+	+	+	+	+	-	-	-	-	-	-	-
Staphylococcus aerues	+	+	+	+	+	-	-	-	-	-	-	-
Bacillus cereus	+	+	+	+	+	+	-	-	-	-	-	-
Streptococcus pyogenes	+	+	+	+	+	-	-	-	-	-	-	-

Table 3.2: MIC result of ethanol garlic BD Type-2

Extract: Methanol garlic BD Type-1

		Concentration of garlic extract										
Organisms	Positive control	100%	90%	80%	70%	60%	50%	40%	30%	20%	10%	Negative control
Vibrio cholerae	+	+	+	+	+	+	-	-	-	-	-	-
Staphylococcus aerues	+	+	+	+	+	+	-	-	-	-	-	-
Bacillus cereus	+	+	+	+	+	+	+	+	-	-	-	-
Streptococcus pyogenes	+	+	+	+	+	+	-	-	-	-	-	-

Table 3.3: MIC result of methanol garlic BD Type-1

Extract: Methanol garlic BD Type-2

		Concentration of garlic extract										
Organisms	Positive control	100%	90%	80%	70%	60%	50%	40%	30%	20%	10%	Negative control
Vibrio cholerae	+	+	+	+	+	+	-	-	-	-	-	-
Staphylococcus aerues	+	+	+	+	+	-	-	-	-	-	-	-
Bacillus cereus	+	+	+	+	+	+	-	-	-	-	-	-
Streptococcus pyogenes	+	+	+	+	+	+	-	-	-	-	-	-

Table 3.4: MIC result of Methanol Garlic BD Type-2

Extract: Ethanol garlic IND

		Concentration of Garlic Extract										
Organisms	Positive control	100%	90%	80%	70%	60%	50%	40%	30%	20%	10%	Negative control
Vibrio cholerae	+	+	+	+	+	-	-	-	-	-	-	-
Staphylococcus aerues	+	+	+	+	+	-	-	-	-	-	-	-
Bacillus cereus	+	+	+	+	+	-	-	-	-	-	-	-
Streptococcus pyogenes	+	+	+	+	+	-	-	-	-	-	-	-

Table 3.5: MIC result of ethanol garlic IND

Extract: Methanol garlic IND

		Concentration of garlic extract										
Organisms	Positive control	100%	90%	80%	70%	60%	50%	40%	30%	20%	10%	Negative control
Vibrio cholerae	+	+	+	+	+	-	-	-	-	-	-	-
Staphylococcus aerues	+	+	+	+	-	-	-	-	-	-	-	-
Bacillus cereus	+	+	+	+	+	-	-	-	-	-	-	-
Streptococcus pyogenes	+	+	+	+	+	-	-	-		-	-	--

Table 3.6: MIC result of methanol garlic IND

3.3 Result of agar diffusion test

Result of agar diffusion test of garlic extract is given below

Extract dilution: 50% extract + 50% distilled water

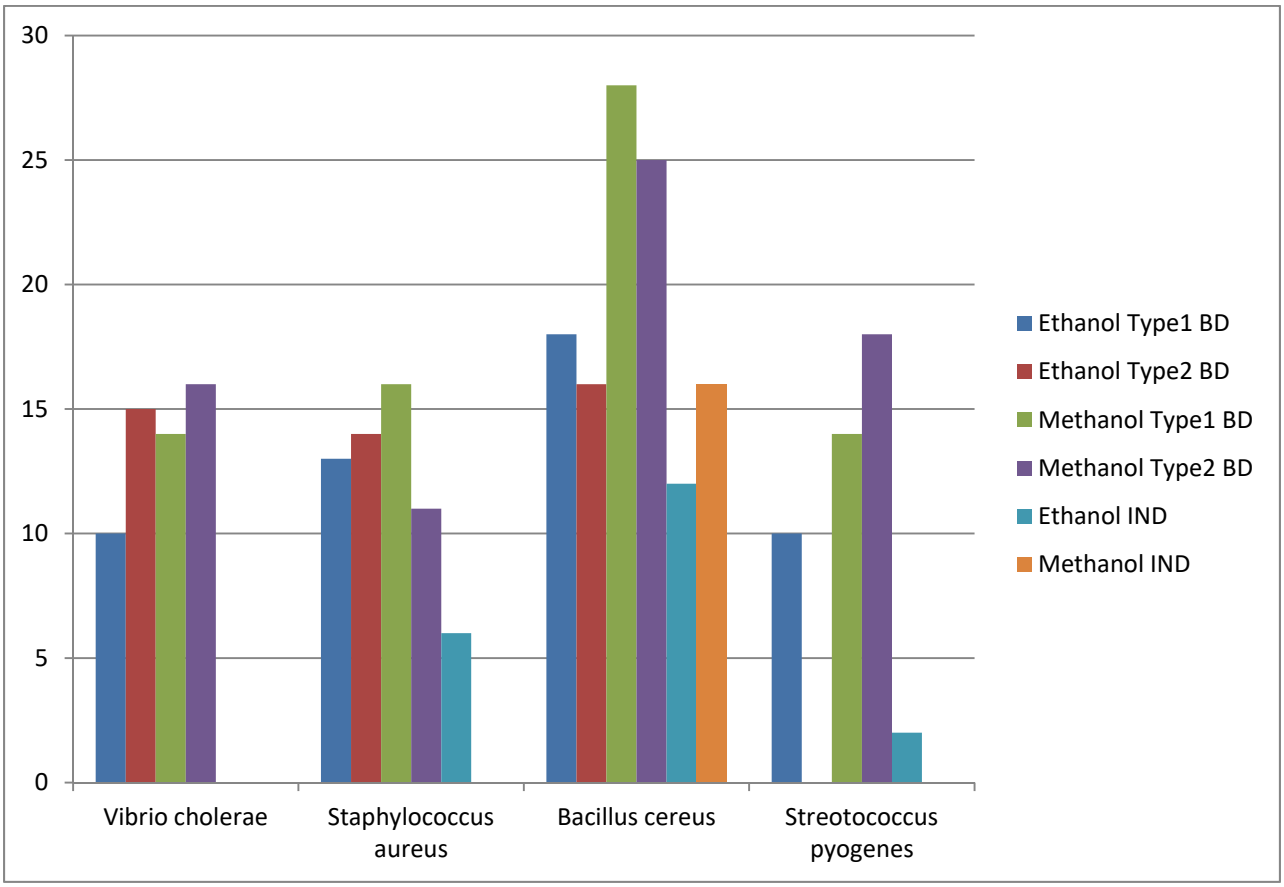


Chart 3.1: Agar diffusion result of 50% extract + 50% distilled water

Microorganism	Zone of inhibition in diameter (mm)					
	Ethanol Type1 BD	Ethanol Type2 BD	Methanol Type1 BD	Methanol Type2 BD	Ethanol IND	Methanol IND
Vibrio cholera	10mm	15mm	14mm	16mm	0mm	0mm
Staphylococcus aureus	13mm	14mm	16mm	11mm	0mm	0mm
Bacillus cereus	18mm	16mm	28mm	25mm	12mm	16mm
Streptococcus pyogenes	10mm	0mm	14mm	18mm	2mm	0mm

Table 3.7: Agar diffusion result of 50% extract + 50% distilled water

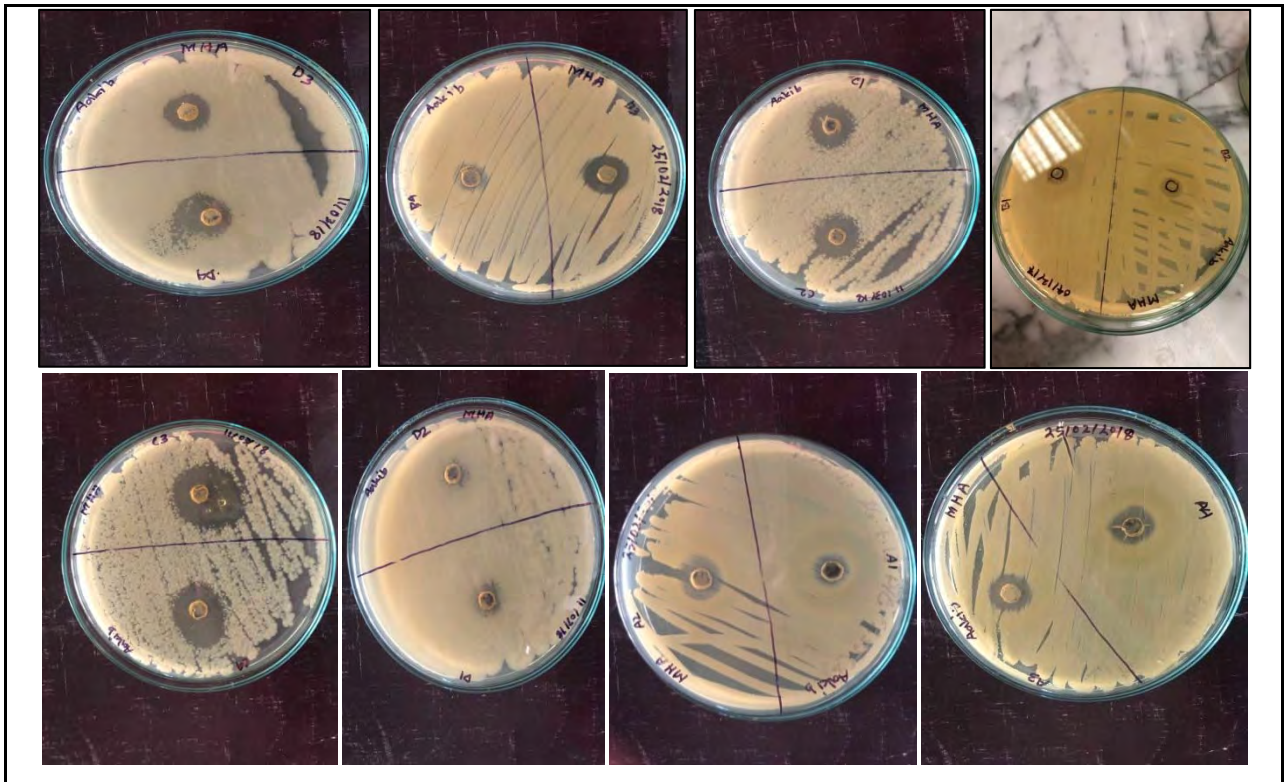


Figure 3.1: Agar diffusion result of 50% extract + 50% distilled water

Extract dilution: 25% Extract + 75% distilled water

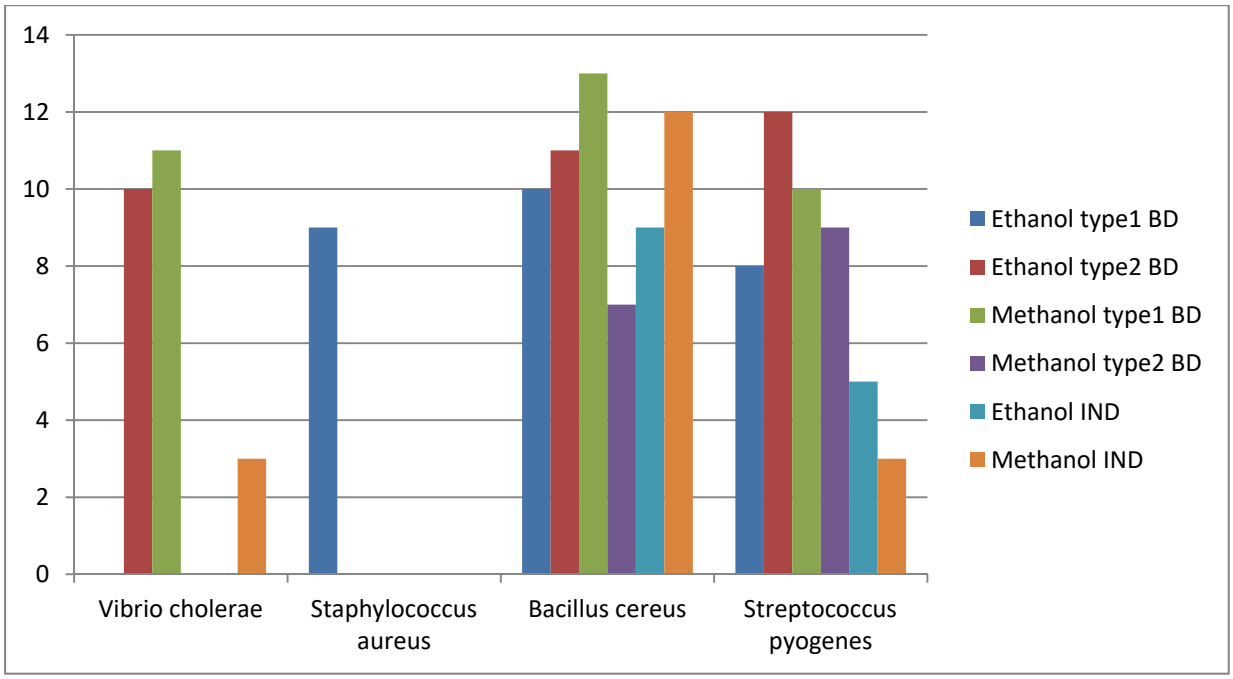


Chart 3.2:Agar diffusion result 25% extract + 75% distilled water

Microorganism	**Zone of inhibition in diameter (mm)**					
	Ethanol Type1 BD	Ethanol Type2 BD	Methanol Type1 BD	Methanol Type2 BD	Ethanol IND	Methanol IND
Vibrio cholerae	0mm	10mm	11mm	0mm	0mm	3mm
Staphylococcus aureus	9mm	0mm	0mm	0mm	0mm	0mm
Bacillus cereus	10mm	11mm	13mm	7mm	9mm	12mm
Streptococcus pyogenes	8mm	12mm	10mm	9mm	5mm	3mm

Table 3.8: Agar diffusion result of25% extract + 75% distilled water

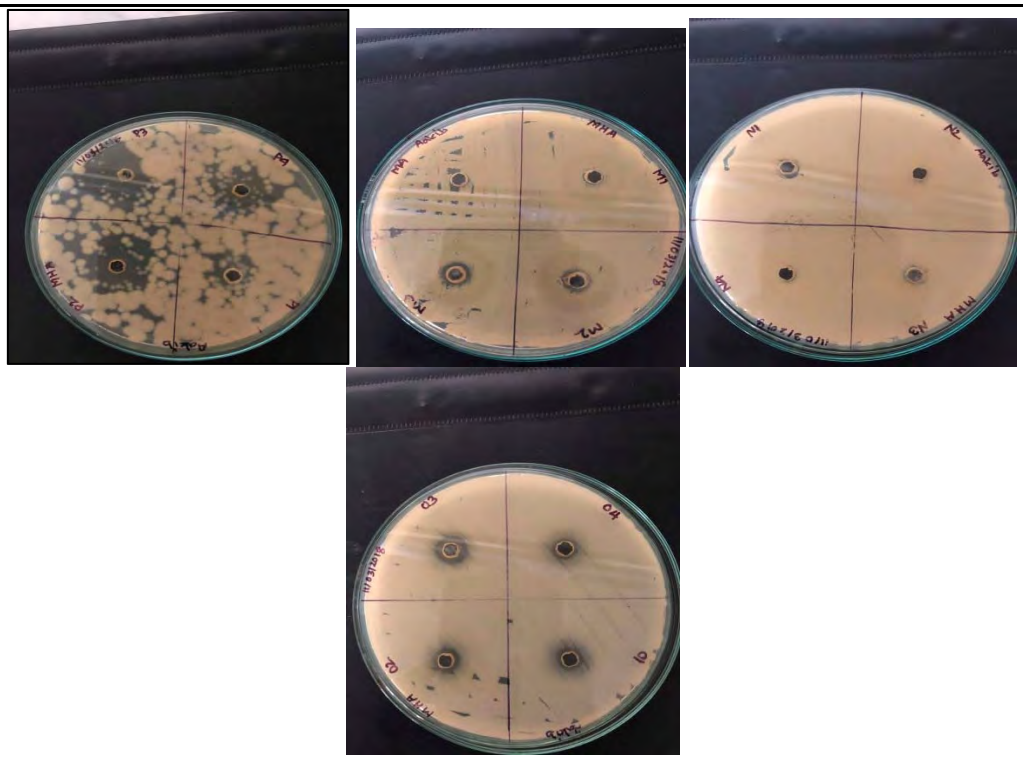


Figure 3.2: Agar diffusion result 25% extract + 75% distilled water

Extract Dilution: 75% Extract + 25% Distilled Water

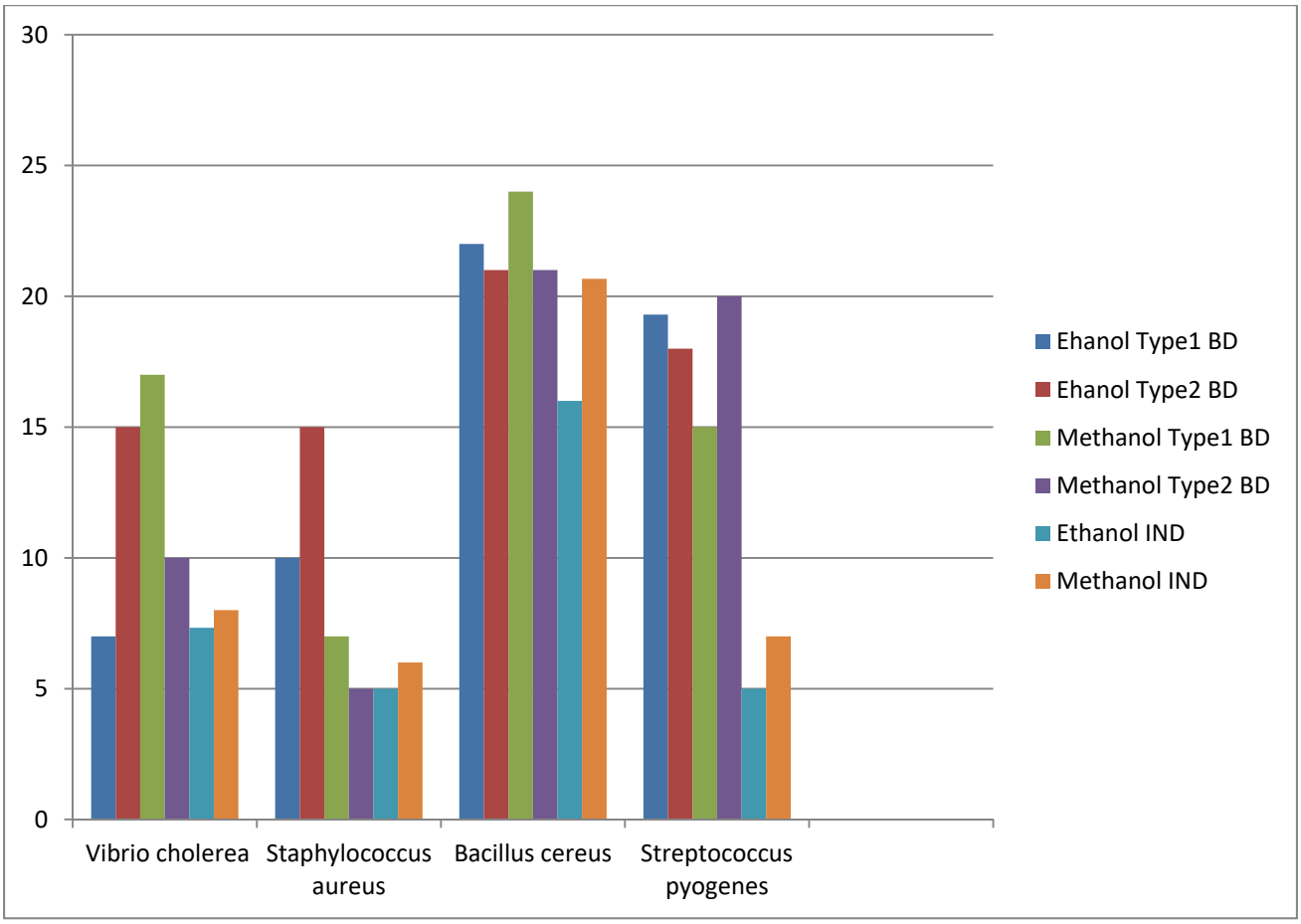


Chart 3.3: Agar diffusion result of 75% extract + 25% distilled water

Microorganism	Zone of inhibition in diameter (mm)					
	Ethanol Type1 BD	Ethanol Type2 BD	Methanol Type1 BD	Methanol Type2 BD	Ethanol IND	Methanol IND
Vibrio cholerae	7mm	15mm	17mm	10mm	7.33mm	8mm
Staphylococcus aureus	10mm	15mm	7mm	5mm	5mm	6mm
Bacillus cereus	22mm	21mm	24mm	21mm	16mm	20.67mm
Streptococcus pyogenes	19.3mm	18mm	15mm	20mm	5mm	7mm

Table 3.9: Agar diffusion result of 75% extract + 25% distilled water

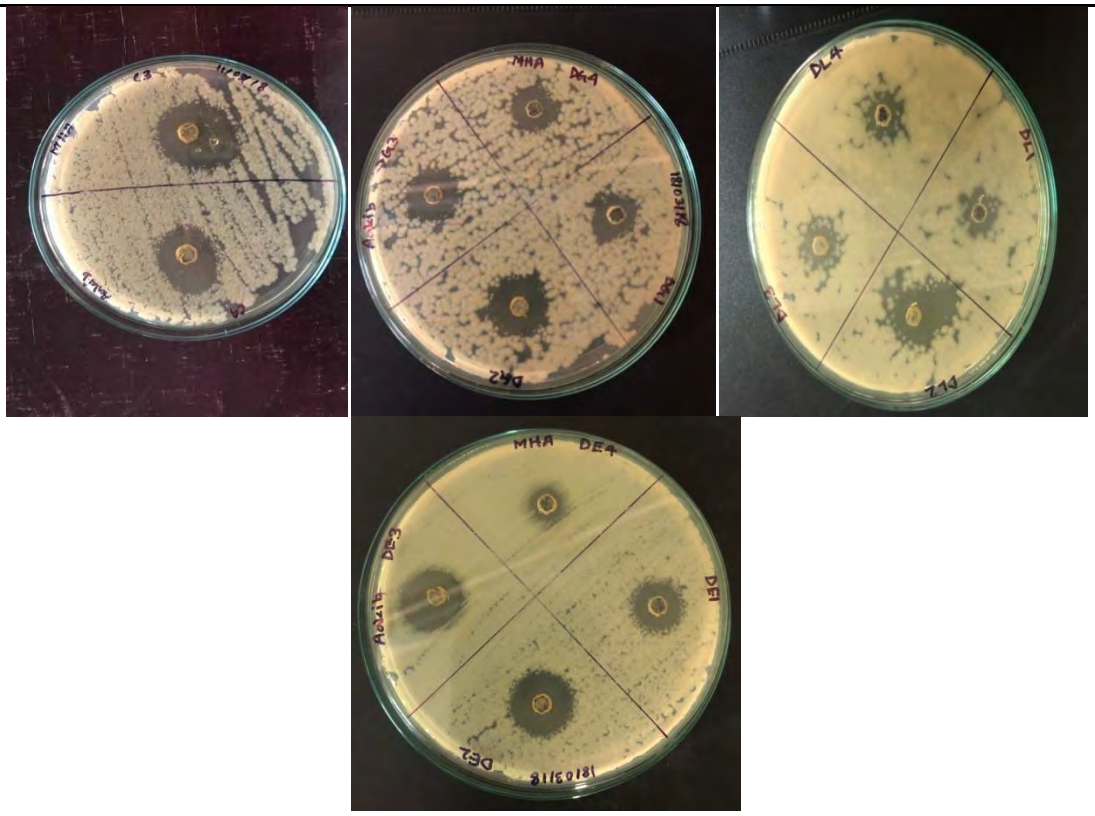


Figure 3.3 : Agar diffusion result of 75% extract + 25% distilled water

Extract Dilution: 75% extract + 25% DMSO

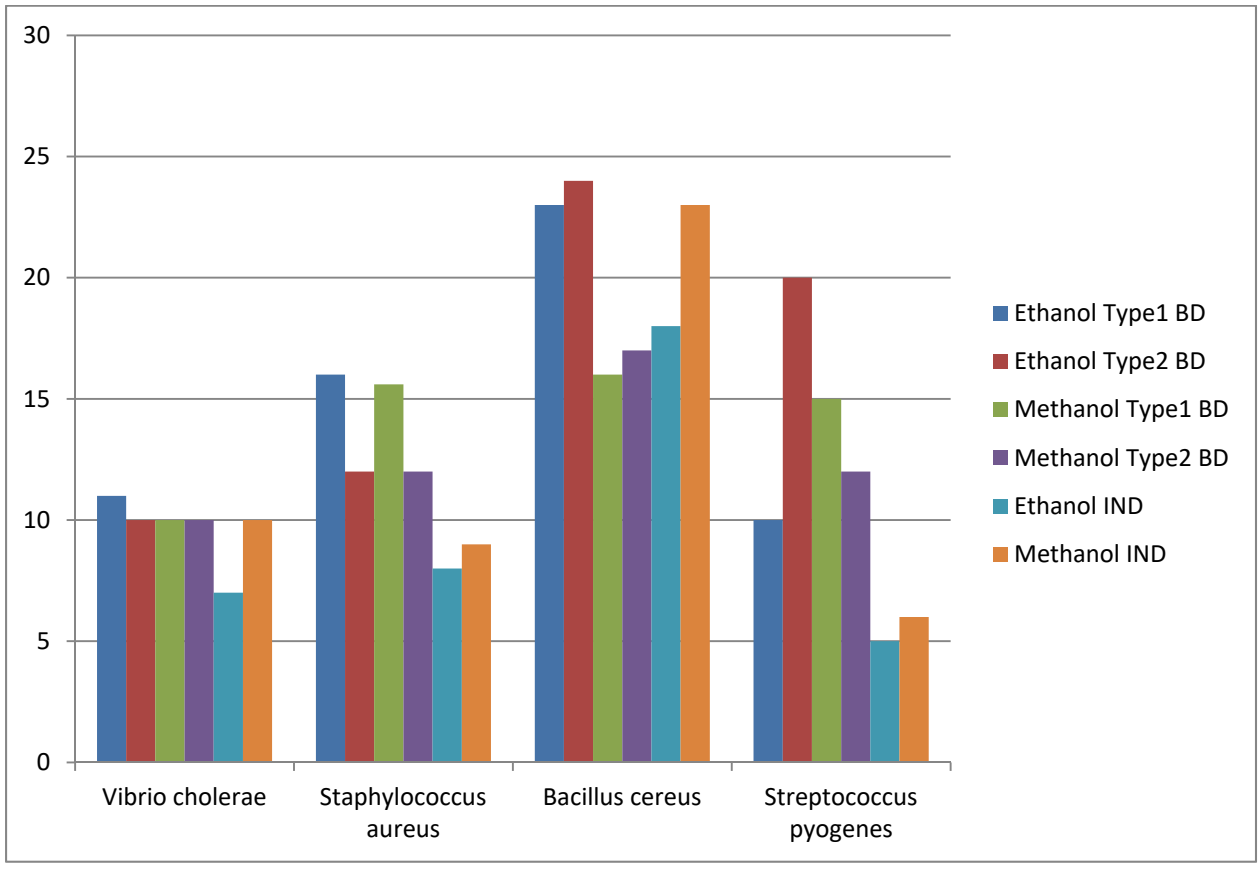


Chart 3.4:Agar diffusion result of 75% extract + 25% DMSO

Microorganism	Zone of inhibition in diameter (mm)					
	Ethanol Type1 BD	Ethanol Type2 BD	Methanol Type1 BD	Methanol Type2 BD	Ethanol IND	Methanol IND
Vibrio cholerae	11mm	10mm	10mm	10mm	7mm	10mm
Staphylococcus aureus	16mm	12mm	15.6mm	12mm	8mm	9mm
Bacillus cereus	23mm	24mm	16mm	17mm	18mm	23mm
Streptococcus pyogenes	10mm	20mm	15mm	12mm	5mm	6mm

Table 3.10:diffusion result of 75% extract + 25% DMSO

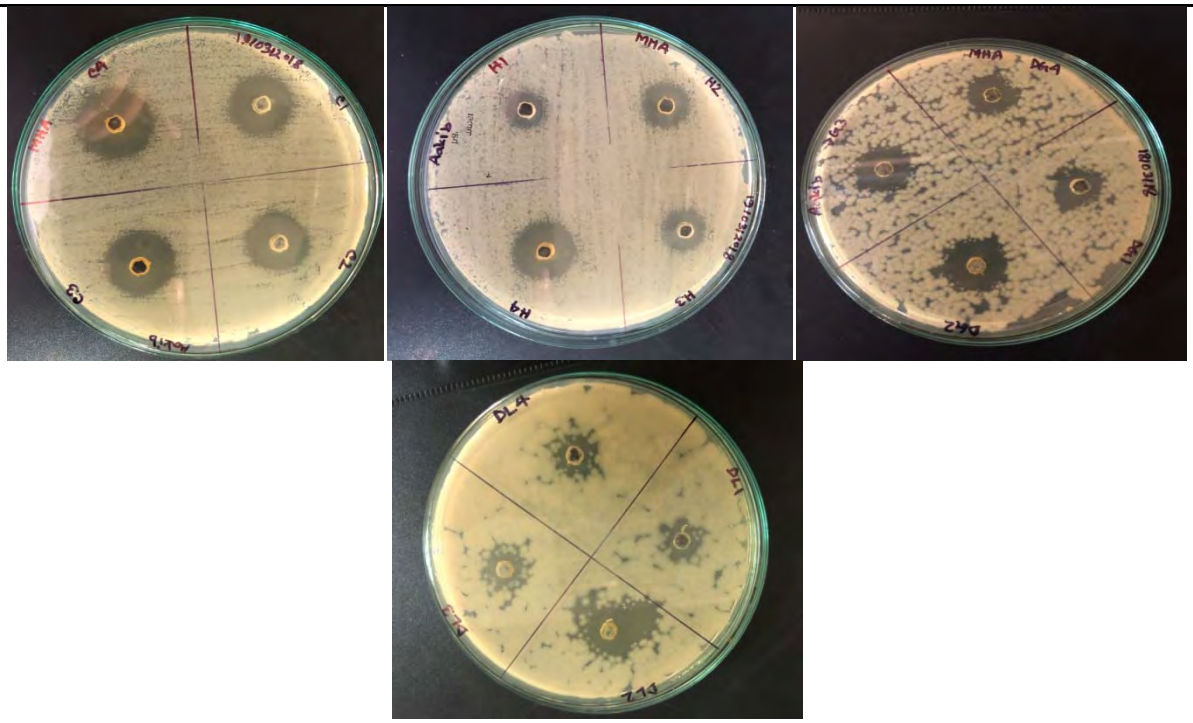


Figure 3.4: Agar diffusion result of 75% extract + 25% DMSO

Extract Dilution: 50% Extract + 50% DMSO

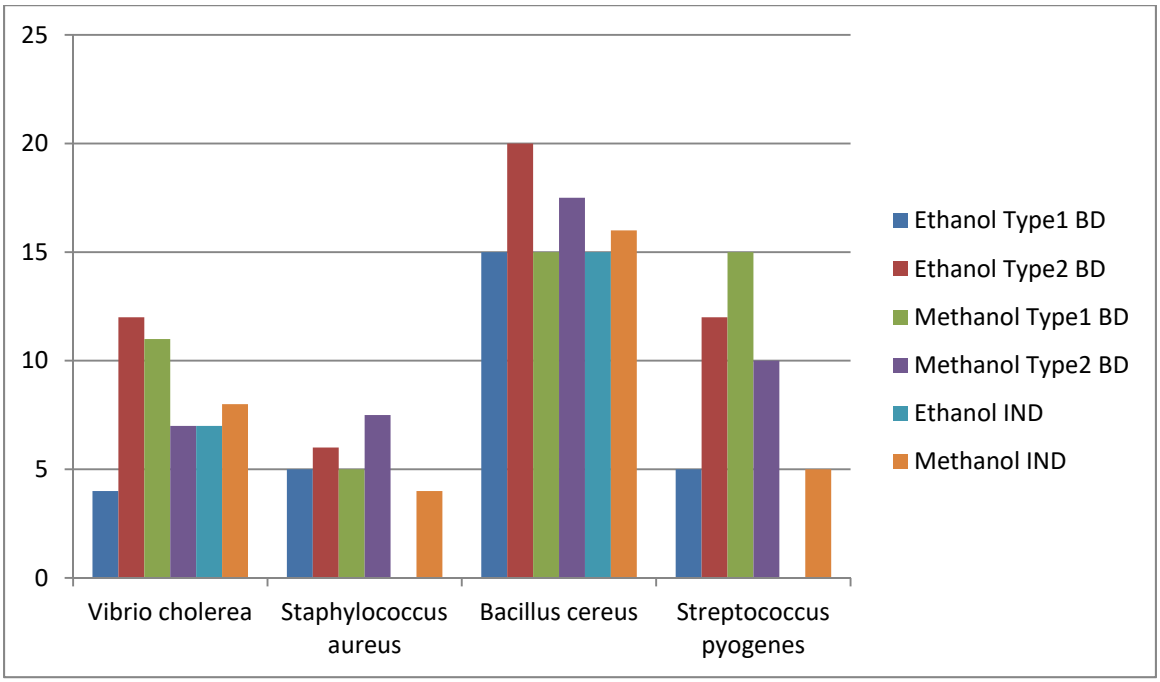


Chart 3.5: Agar diffusion result of % extract + 50% DMSO

Microorganism	**Zone of inhibition in diameter (mm)**					
	Ethanol Type1 BD	Ethanol Type2 BD	Methanol Type1 BD	Methanol Type2 BD	Ethanol IND	Methanol IND
Vibrio cholerae	4mm	12mm	11mm	7mm	7mm	8mm
Staphylococcus aureus	5mm	6mm	5mm	7.5mm	0mm	4mm
Bacillus cereus	5mm	20mm	15mm	17.5mm	15mm	16mm
Streptococcus pyogenes	5mm	12mm	15mm	10mm	0mm	5mm

Table 3.10:Agar diffusion result of % extract + 50% DMSO

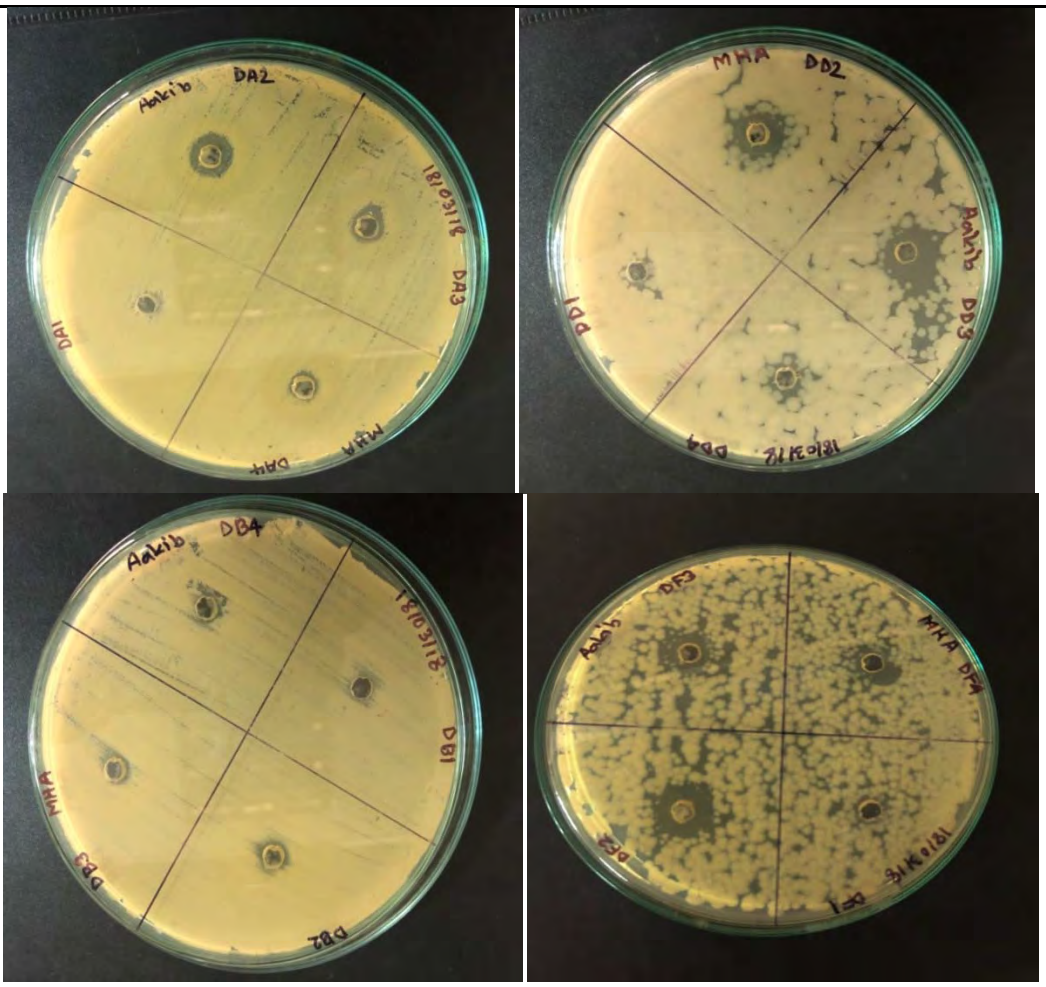


Figure 3.5: Agar diffusion result of % extract + 50% DMSO

Extract Dilution: 25% extract + 75% DMSO

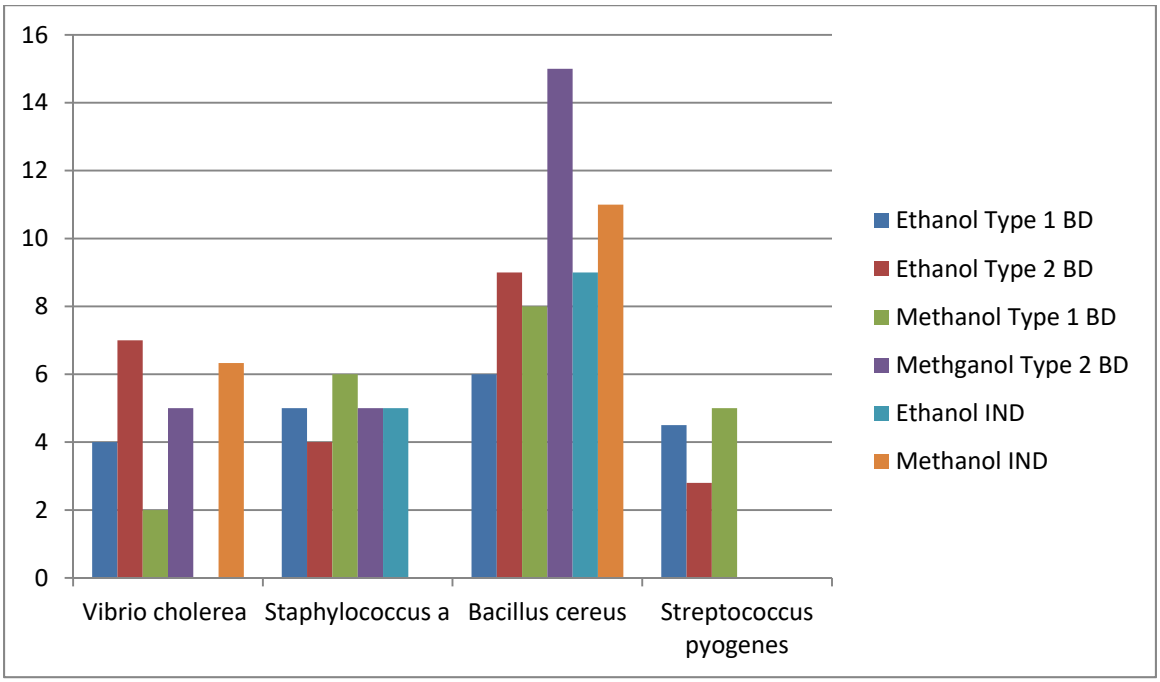


Chart 3.6: Agar diffusion result of 25% extract + 75% DMSO

Microorganism	Zone of inhibition in diameter (mm)					
	Ethanol Type1 BD	Ethanol Type2 BD	Methanol Type1 BD	Methanol Type2 BD	Ethanol IND	Methanol IND
Vibrio cholerae	4mm	7mm	2mm	5mm	0mm	6.33mm
Staphylococcus aureus	5mm	4mm	6mm	5mm	5mm	0mm
Bacillus cereus	6mm	9mm	8mm	15mm	9mm	11mm
Streptococcus pyogenes	4.5mm	2.8mm	5mm	0mm	0mm	0mm

Table 3.11:Agar diffusion result of 25% extract + 75% DMSO

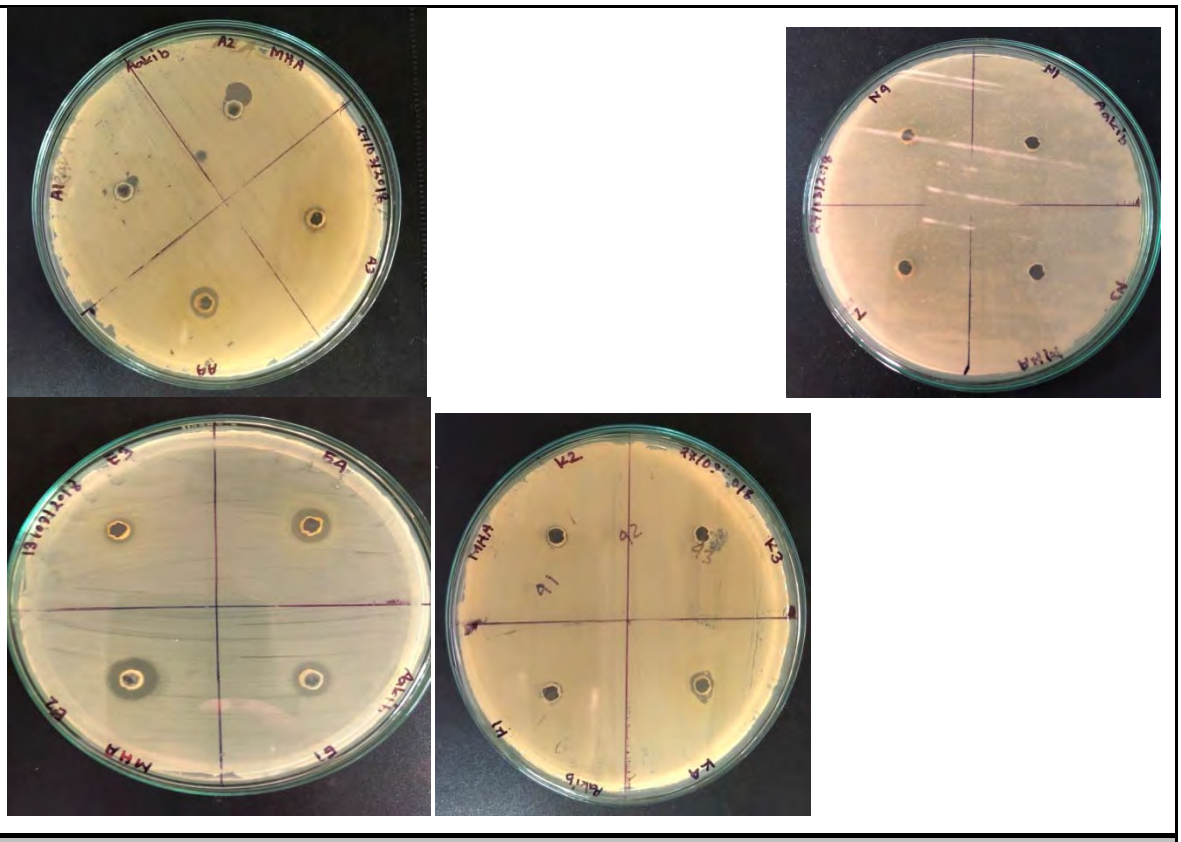


Figure 3.6: Agar diffusion result of 25% extract + 75% DMSO

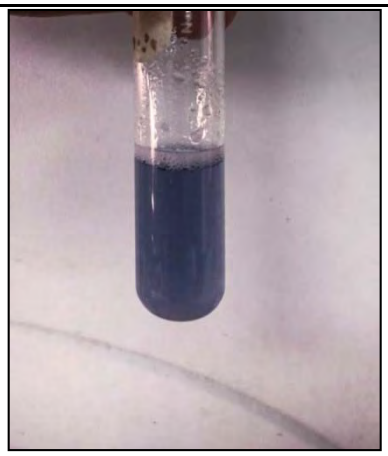
The results suggest that there was a significant effect of the garlic extract on inhibition of the growth of the above-mentioned bacteria.

3.4 Result of Phytochemical test

From the result of qualitative phytochemical screening of Bangladeshi and Indian garlic extract it was found to contain proteins, tannis and phenols, glycosides, saponins and alkaloids while carbohydrates, flavonoids are absent. Triterpenoids is present in Bangladeshi garlic but absent in Indian garlic.

Phytochemical Constituent	Garlic BD	Garlic IND
Protiens	+	+
Tannis & Phenols	-	-
Glycosides	+	+
Saponins	+	+
Flavonoids	-	-
Triterpenoids	+	-
Alkaloids	+	+

Table 3.12: Phytochemical profile of garlic (*Allium sativum*)



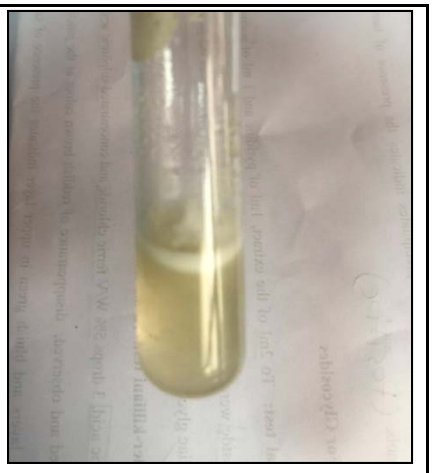
A

B

Figure 3.7: Phytochemical test result (A) positive result of Biuret's test and (B) positive result of Lead acetate test

A

B



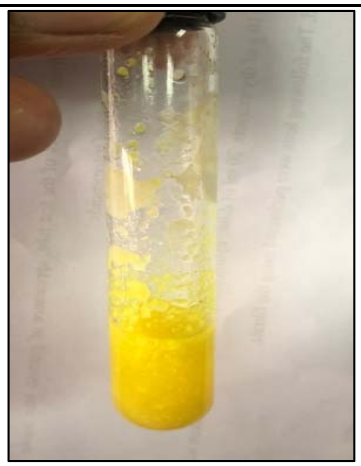
C

Figure 3.8: Phytochemical test result(A) positive result of Foam test, (B) negative result of Shinoda test and (C) positive result of Keller- killani test

A

B

Figure 3.9: Phytochemical test result (A) positive result of Salkowaski test and (B) positive test result of Wagener's test



A

B

Figure 3.10: Phytochemical test result (A) positive result of Hager'stest and (B) positive test result of Dragendroff's test

Discussion

The growing population concern about health problems has recently led to the development of natural antimicrobials to control microbial diseases. Medicinal plants and spices are one of the most commonly used natural antimicrobial agents in foods and have been used traditionally for thousands of years by many cultures for controlling common health complications. Natural plant product based antimicrobials drug discovery attained paramount importance as newly discovered drugs are likely to be effective against multi drug resistant microbes.

According to various repost garlic has traditional and medical applications as an anti-infective agent (Ross ZM, 2001). Fresh and dried In vitro evidence of the antimicrobial activity of fresh and freeze dried garlic extracts against many bacteria (Rees LP, 1993), fungi and viruses (Weber ND, 1992) supports these applications. Allicin, the active ingredient of garlic, acts by partially inhibiting DNA and protein synthesis and also totally inhibiting RNA synthesis as a primary target (Eja ME, 2007). Organosulfur compounds and phenolic compounds have been reported to be involved in the garlic antimicrobial activity (Johnson M, 2011).

This study was to prove the activity of garlic against *Vibrio cholera*, *Staphylococcus aureus*, *Bacillus cerues* and *Streptococcus pyogenes* and to compare the activity of garlic produced in Bangladesh and India.

The result of agar diffusion assay shows that there is a significant zone of inhibition of all strain and Garlic produced in Bangladesh has a grater zone of inhibition over garlic produced in India. 75% and 50% extract showed the most effect against the all strains of selected bacteria. However, the 25% of Bangladeshi garlic extract has less effective against the strains.

According to the table *Bacillus cerues* showed the highest zone of inhibition and Staphylococcus aureus showed the least zone of inhibition for the Bangladeshi garlic extract.

On the other hand, Indian garlic extract were less effective against the selected strains of bacteria. According to the table most of the strain showed no zone of inhibition.

From figure we can see that a bigger zone of inhibition by the extract of garlic produced in Bangladesh than Indian garlic.

From the result of this study, it was found that the crude ethanolic and methanolic extract of the Bangladeshi garlic was very much effective against the sensitive pathogens and it was more effective than the Indian garlic. From other studies on garlic extract, it was learned that it is effective against broad spectrum of bacteria, mold and fungi too. Therefore, By further research Bangladeshi garlic extract could

be one of the antimicrobial alternate to the antibiotics to decrease the use of antibiotics. We can also say that Bangladeshi garlic can improve our immune system than Indian garlic. The result of this study is based on *in vitro* test with the organisms. *In vivo* test with animal model has to be done first before implication.

Probably, the antimicrobial activity of the garlic extract because of the combined effect of the Allicin which inhibits the RNA and partially inhibits the DNA and Organosulfur. From the result of phytochemical screening of Bangladeshi and Indian garlic we saw that both extract has various compounds like Proteins, tannins, phenols, saponins and alkaloids. However Bangladeshi garlic has triterpenoids but on the other hand Indian garlic does not have tritenpenoids. From the various studies we came to know that triterpenoids has many health benefiting activities like cancer prevention We cannot determine that because of the absence of trierpenoids in Indian garlic is the reason for poor antibiotic activity against selected microorganism. It would be great if the exact antimicrobial compounds could be identified from the crude garlic extract. Accordingly, the compounds need to be identified and purified using high performance liquid chromatography (HPLC) or other high throughput technique. If the major antimicrobial compound is identified, the compound could be further tested *in vitro* using mammalian cell line and *in vivo* test using an animal model.

Conclusion

The ethanolic and methanolic extract of Bangladeshi garlic could be source to obtain a new alternative to improve our immune system. We should increase the consumption of Bangladeshi garlic instead of Indian garlic which has less health benefits to our body. The phytochemical analysis of the Bangladeshi garlic and Indian garlic extract helped us to determine and characterization its active compounds. Further chromatographic screening will help us to determine the quantitative analysis and compounds of the garlic. However, we need to find out the side-effect and pharmalogoical kinetics of the Bangladeshi garlic before implementing it as a well established antibiotic alternative Moreover; these plants extract should be investigated *in vivo* to better understand their safety, efficacy and properties.

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Appendix

Appendix-1

Media composition:

a. Nutrient Agar:

Component	Amount (g/L)
Peptone	5.0
Sodium chloride	5.0
Beef extract	3.0
Agar	15.0
Final pH	7.0

b. Nutrient broth:

Component	Amount (g/L)
Peptic digest of animal tissue	5.0
Sodium chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Final pH	7.4±0.2 at 25ºC

C.Saline

Component	Amount (g/L)
Sodium Chloride	9.0

d.Muller Hinton Agar:

Component	Amount (g/L)
Beef, dehydrated infusion form	300
Casein hydrolysate	17.5

Starch	1.5
Agar	17.0
Final pH	7.3± 0.1 at 25°C

e. Mueller Hinton Broth (Oxoid, England)

Component	Amount (g/L)
Beef, dehydrated infusion form	300
Casein hydrolysate	17.5
Starch	1.5

Appendix 2

Reagents

a. Wagner's reagent
To a reagent bottle add 2 gram of iodine and 6 grams of potassium iodide (KI), dissolve this with 100cm^3 of water and mix well. Store the reagent at room temperature.

B. Hager's reagent
To a reagent bottle add 1 gram of piric acid and dissolve this with 100cm^3 of water

C. Dargendroff's reagent
To a reagent bottle add 0.5 grams of bismuth nitrate and mix this with 10ml of distilled water. The mixture should be like a suspension. Then add 10ml of concentrated hydrochloric acid and stir the mixture. After that add 4 grams of dissolved potassium iodide in the mixture and observe for dark orange solution. Then store the reagent at room temperature.

Appendix – 3

Instruments:

The important equipment used through this study are listed below

Autoclave	Model: WIS 20R Daihan Scientific Co. ltd, Korea
Sterilizer	Model no: NDS-600D, Japan
Balance machine: Adam	UK
Freezer (-4^{o} C)	Samsung
Incubator	Model-0SI-500D, Digi system Laboratory Instruments Inc. Taiwan
Laminar Airflow Cabinet	Model-SLF-V, vertical, SAARC group Bangladesh
Micropipettes	Eppendorf, Germany
Oven (Universal drying oven)	Model: LDO-060E , Labtech, Singapore
Refrigerator	Samsung
Vortex mixture	Digi system Taiwan, VM-2000
Shaking Incubator WiseCube	Shaking Incubator WiseCube
Grinder	Philips