

**A Project Report**

**On**

**FORMULATION OF HEALTH DRINK HAVING  
ANTIBACTERIAL ACTIVITY.**

**Submitted By**

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**2023 -2024**



Shri Swami Vivekanand Shikshan Sanstha's

**VIVEKANAND COLLEGE, KOLHAPUR  
(EMPOWERED AUTONOMOUS)**

**PG DEPARTMENT OF MICROBIOLOGY**

**CERTIFICATE  
OF  
RESEARCH PROJECT COMPLETION**

This is to certify that **Ms. Namrata Laxman Bhosale** studying in M. Sc. part II Microbiology at Vivekanand College, Kolhapur (Empowered Autonomous) has sincerely completed research project work entitled **“FORMULATION OF HEALTH DRINK HAVING ANTIBACTERIAL ACTIVITY”** during academic year 2023-24.

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Dr. G. K. Sontakke

Head of the Department



“Dissemination of education for Knowledge, Science and culture”  
- Shikshanmaharshi Dr. Bapuji Salunkhe

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<b>Sr. No.</b>	<b>Title</b>	<b>Page No.</b>
1.	Introduction	1-6
2.	Methods and Materials	7-14
3.	Result and Discussion	15-30
4.	Summary and Conclusion	31



## 1.0 INTRODUCTION

Health drink is an important part of the healthy diet. There are various number of commercial health drinks available in the market such as Horlicks, Protinex, Bournvita, Boost etc. These commercial health drinks contain all artificial ingredients and are costly in nature. These drinks have only nutritive value as well as many people cannot consume it due to high sugar content and calories. Moreover, these commercial drinks do not carry any antimicrobial activity. Hence, our study was aimed to use natural ingredients for preparation of health drink which will be economically, affordable, nutritious and have antimicrobial activity.

India is rich with different natural substance having antimicrobial activity and these substances are used in diet every day. The examples of such substances are ginger, turmeric, garlic, black pepper, onion, ajwain, mustard seeds, coriander, mint leaves, lemon and honey etc [Jessica B., 2023]

The antimicrobial agent is a substance which have ability to kill different types of microorganisms. The antimicrobial agent may be antiviral, antifungal, antibacterial. These agents have various types of mode of action to suppress the growth of microorganisms [Issan V., 2021].

Antimicrobial activity testing of a substance can be done by paper disc method like agar well diffusion method [Magaldis M., 2004]. In this study antimicrobial activity of natural substance such as lemon and honey were evaluated by agar well diffusion method.

Honey Bees use nectar from flower and produce sweet food known as honey. Due to the low water activity, most microorganisms do not grow and survive in honey [Molan 1992]. Quality of honey depends on osmolarity [Kwakman and zeet, 2012]. Low pH [Karabagis et. al., 2014] antimicrobial factors [Molan, 2001] hydrogen peroxide [White et al., 2020].

Phenolic compound of honey also contributes to antimicrobial activity [Estevin et. al., 2008]. The honey bee dehydrate and their own compound modify the



nectar. This nectar mature and develop into honey. It is a concentrated viscous aqueous sugar solution. It generally consists of glucose [30%] fructose [40%] sucrose [5%] water [20%]. Small quantities of disaccharides (e.g. trehalose, isomaltose, maltose). The composition of honey differs due to seasons, botanical sources and nectars (Saudi J., 2020).

The different type of amine acid protein, minerals, enzymes (Invertase & glucose oxidase) polyphenols and vitamins are also present in Honey. The properties and composition of honey also depend on the surrounding environment of the hive and metabolic activities of honey of honey bees. For e.g. the collection of nectar can be either multiflora (Multiple species of plant) or monoflora (Single species of plant) which can give a unique taste and different properties (Bevord, 1996) to honey.

The enzyme glucose oxidase is necessary to produce ( $H_2O_2$ ) the enzymatic oxidation of glucose produce gluconic acid & ( $H_2O_2$ ) species. Honey is lack of free water, due to this there is no activity of enzymes in raw. Honey, hence needs to dilute to initiate the peroxide dependent antimicrobial mechanism. The antimicrobial activity of honey is essential to recover the wound because it provide an effective hydrated barrier between the wound site and external environment. Burns trauma and chronic wounds can also be treated by honey.

In tissue engineering, application of honey derived and honey inspired products are used with combination of other biomaterials. It is also used in other clinical situation outside wound care. Honey is tested against different bacterial strains it. Results indicated that more effective activity against *Staphylococcus aureus*, *Salmonella spp*, *Pseudomonas*, *Proteus*, *Proteus mirabilis* & *E. coli*. The cytotoxicity of  $H_2O_2$  is effective against each bacterial strain. The  $H_2O_2$  itself is not antimicrobial. The antimicrobial activities occur due to reactive hydroxyl free radical which produces from catalytic action of traces of metal ions from the pathogens cell.



Average pH of honey is 3.9 but can range between 3.4 and 6.1. Hence honey is acidic in nature. This acidity is initially produced from gluconic acid and gluconolactose due to enzymatic breakdown of glucose. There are some good antimicrobial activities of honey against Rubella Virus when treated in vitro infected monkey kidney cell culture [Akterm J., 1996].

Honey is used as traditional medicine to cure wound. A range of both Gram negative and gram positive bacteria are inhibited by honey.

Honey is used as an important source of carbohydrate and natural sweetener. Honey is rich in sugar, minerals, organic acid, enzymes, vitamins and proteins. Gastrointestinal disorders such as peptic ulcer, gastric ulcer and gastroenteritis are prevented by consumption of honey. It also gives probiotic effects thus improve gastrointestinal health. It is directly absorbed in blood stream. Without digestion. Is used in control & treatment of wounds, diabetes and treatment of asthma and also cardiovascular neurological and gastrointestinal disease. Honey is applied daily in the eyes. It improves the eye, sight. Moreover, honey is regarded as useful in prevention of eye contract. Honey is used as topical ointment. Honey is also used for treatment of baldness, wound healing, contraception, sore throat, eye diseases. Honey as one of the best medicines in treatment of tuberculosis. Diluted honey treated urinary tract infection.

Lemon is an important medicinal plant of family Rutaceae (J.V. Ewansiha, 2020). This citrus fruit is acidic fruit which contains healthy nutritional content that works wonder for the body. It consists of abundant source of vitamin C and wide range of essential nutrients required for our body (AI- Rafidain Dent J. 2000).

It is cultivated mainly for its alkaloid activity which have antimicrobial potential and anticancer activities in crude extract of leaves stem, root juice and flower of lemon. Antibacterial antifungal, antidiabetic anticancer and antiviral citrus is because of flavonoids large spectrum of these biological activities. Citrus lemon is an ever-green plant native to Asia. It is popularly utilized



for its juice pulp & also for peels. In cooking dishes, it is world widely used because its juice provides unique sour test. It is also rich in citric acid (pH 2-3). Lemon is used to make lemonades, cocktails, and beverages. It has number of medicinal values. [Konwar et al 2001]. It is easily available and common in use. Lemon juice has significant antimicrobial activities against different types of pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella*, *Pseudomonas aeruginosa*, *Salmonella*, *Proteus mirabilis*, *Proteus* & *Candida albicans*

According to chemical research lemon juice consists of flavonoids, citric acid, alkaloid, ascorbic acid. These ingredients increases. The nutritional value, taste and appearance of product (Giuseppe G et. al., 2007). Flavonoids are mainly present in citrus .fruits as their glycerol derivatives. When flavonoids are present in citrus. Juice in large amount it acts as antioxidant in various biological systems. Phenolic compound are also present in lemon juice which showed to be powerful antioxidant and radical scavengers (Robinson R., 2000).

According to phytochemical analysis lemon juice also consists of phytochemicals such as tannins, anthraquinones, steroids, cardiac glycoside, terpenes, resins and saponins, fatty acids, aliphatic and aromatic hydrocarbons, ethyl acetate etc. Alkaloid compound function as anticancer, antibacterial flavonoid compound. These include phenol compounds that have ability to denaturize bacterial cell proteins and also damage the cell membranes. (Prastiwi S. et al 2013). Saponin are antimicrobial compounds which are responsible to damage bacterial cell membranes. Citrus juice contains vitamin C which is useful as antioxidant. The main important in gradient in lemon juice is organic acid in the form of citric acid provide that acidity. pH is one of the important factor that can inhibit bacterial growth which can cause the internal pH of bacterial cell to decrease and inhibit bacterial cell growth. (Berti P.L. 2015). Lemon is medicinal plant which contain various natural resources used as agrochemicals, flavours, fragrances, dye, and food additive.



That are beneficial to health. It also contains various substances including, fiber, vitamin C, carbohydrates, calcium, folate, potassium, thymine, vitamin B6, niacin, phosphorous, vitamin A, copper, magnesium, riboflavin. These substances are needed for our body. Some compounds present in citrus juice are able to provide additional protection for the body against the chronic disease. According to previous pharmaceutical study, lemon juice has antinflammatory insect repellent, anticancer, antioxidant, antimicrobial, reproductive, cardiovascular, gastrointestinal, analgesic antidiabetic, immunological respiratory and many other pharmaceutical effect. (AI. Snafi, A.E. 2016). Vitamin C present in lemon also helps in the process of iron absorption in the body.

Researcher's suggest that vitamin C and antioxidant's both are nutritious and beneficial for heart health and helps to prevent heart diseases and stroke. However some amount of fibers present in lemon can also significantly lower some risk factors for heart disease. Consuming lemon juice can help to reduce high blood pressure. Lemon contains two plant compounds, such as hesperidin and diosmin which are known to lower cholesterol.

Lemon is immunity boosting fruit, as it contains high amount of vitamin C antioxidant. It helps boost the immune system again germs. A glass of hot water with lemon juice and honey and a large spoonful of honey can help

Lemon contains a high amount of soluble fibres that helps maintain regular bowel movements and also improves digestion. The main fibre present in lemon named pectin helps to improve gut system/health by increasing the digestion rate of starch and sugar ayurvedic medicine believes that drinking a glass of water with lemon juice can improve your digestion system. High vitamin C content in lemon can boost testosterone levels in men.

Fully squeezed lemon in glass of lukewarm water with tablespoon of honey can be magic drink for many as this drink may aid in weight loss. Lemon contains compound called pectin. It is a fibre that expands after ingestion making you feel



full sooner and longer. It will prevent you from snacking on unhealthy foods that will lead to weight gain. Lemon and lemon juice are rich in antioxidants and vitamin C that helps fight against deadly diseases like cancer, components present in lemon-limonene and narinegenin have anti-cancer properties.

Vitamin C is essential vitamin for teeth and gums, hence lemon with rich source of vitamin C is beneficial for oral disorders. Lack of vitamin leads to swollen gums, bleeding gums. Lemon juice may have pain killing effects when applied locally to areas where there is toothache. It may also help to reduce inflammation.

Lemon contains high concentrations of vitamin C, which is required to generate collagen. Collagen gives our skin plum and youthful look. It helps to reduce the fine lines on the face and make your skin clear.

Daily intake of lemon along with 30-60 minutes of brisk walking can keep your high blood pressure in check. Those who consumed lemon, showed reduced sign of hypertension as opposed to those who did not. Lemon oil may improve your mood by calming down anxiety and boosting your spirits.

High level of citric acid present in fresh fruit as well as lemon juice concentrate can improve urinary citrate levels two fold, without altering the total urinary volume. Lemon juice concentrate can prevent kidney stones by forming urinary citrate which acts as preventive method for crystal development.

Consumption of lemon cough drops when suffering from throat infection will lessen the bacterial effects and bring about relief. Lemon is high in vitamin C which boosts collage and improves hair growth by stimulating hair follicles. When suffering from hair loss. Mix two tablespoons of lemon juice with aloe vera gel and apply this to your scalps 30 minutes before your bath.



## **2.0 Methods and Materials**

### **2.1 Collection of Sample**

#### **Lemon – *Citrus Lemon***

Fresh citrus lemon variety of lemon were collected from Kolhapur market and packed in to polythene bags and bought to the laboratory and kept in refrigerator.

#### **Honey- *Apis dorsata***

The different variety of honey are available in the market. For study, Dabur honey was purchased from Kolhapur market.

Purity of honey was checked in the laboratory by the following process: One tablespoon of honey was mixed with 10 ml water and stirred with glass rod. If honey get settled at bottom, it indicates that honey is pure.

### **2.2 Bacterial cultures**

Following bacterial pathogen cultures available in our laboratory where used in the study

- *Salmonella typhi*
- *Escherichia coli*
- *Proteus spp.*
- *Proteus mirabilis*
- *Pseudomonas aeruginosa*
- *Staphylococcus aureus*

The microorganisms were reconstituted by sub culturing on to freshly prepared nutrient agar plates. The incubated at 37<sup>0</sup> Celsius for 24 hours, after which their purity was confirmed by Gram staining.



## 2.3 Culture media

Nutrient agar medium was used for cultivation and checking the antibacterial activity of lemon and honey.

## 2.4 Sample Preparation

### 2.4.1 Lemon

#### (A) Lemon Juice Extraction

- 1) Selected lemon was washed with sterile distilled water. Surface of lemon was sterilized with 75% alcohol.
- 2) After drying the surface lemon was cut with the help of sterile knife. For this knife was dipped in alcohol then flamed out, after cooling lemon was cut into equal pieces
- 3) Seeds of lemon were remove with the tip of knife
- 4) The lemon juice was collected aseptically in sterile flask

#### (B) Dilutions of lemon juice

Table -1 Dilution of lemon juice

Sr. No.	Dilution of Lemon (%)	Sterile Potable Water (ml)	Lemon Juice (ml)
1	1	9.9	0.1
2	10	9	1
3	20	8	2
4	30	7	3
5	40	6	4
6	50	5	5
7	60	4	6
8	70	3	7
9	80	2	8
10	90	1	9
11	100	-	Pure



(C) Study of antibacterial activity of lemon juice

***Staphylococcus aureus***

Study of antibacterial activity of lemon juice against *Staphylococcus aureus*

1. Preparation of suspension of *Staphylococcus aureus*  
A loop full of freshly grown *Staphylococcus aureus* was added in to sterile saline to prepare thick suspension of organism
2. Preparation of nutrient agar  
80ml nutrient agar was prepared & sterilized by autoclaving
3. Preparation of seed agar  
80 ml sterile molten cooled nutrient agar medium was added with suspension of *Staphylococcus aureus* & plates were poured
4. Solidification of medium  
Plates were allowed to solidify
5. Preparation of Wells  
Wells of approximately 10 mm diameter were cut using the sterile cork borer.
6. Addition of lemon dilutions  
Each dilution of lemon was added in a separate well and proper labelling was done. In one well sterile potable water was added and it was labelled as control
7. All plates were kept in refrigerator at 4° c for 15 mints
8. After that plates were incubated at 37°c for 24 hrs.
9. After incubation plates were examined for inhibition zone around each well

Similar procedure was followed for all other pathogens

- *Salmonella typhi*
- *Escherichia coli*



- *Proteus spp.*
- *Proteus mirabilis*
- *Pseudomonas areuginosa*
- *Klebsiella pneumoniae*

#### 2.4.2 Honey

The bottle of honey was opened aseptically and different dilutions of honey were done using sterile potable water as diluent.

Table -2 Dilutions of honey

<b>Sr. No.</b>	<b>Dilutions of Honey (%)</b>	<b>Sterile Potable Water (ml)</b>	<b>Honey (ml)</b>
1	1	9.9	0.1
2	10	9	1
3	20	8	2
4	30	7	3
5	40	6	4
6	50	5	5
7	60	4	6
8	70	3	7
9	80	2	8
10	90	1	9
11	100	-	pure



### *Staphylococcus aureus*

To study antibacterial activity of honey against *Staphylococcus aureus*

1. Preparation of suspension of *Staphylococcus aureus*

A loop full of freshly grown *Staphylococcus aureus* was added in to sterile saline to prepare thick suspension of organism

2. Preparation of nutrient agar

80ml nutrient agar was prepared & sterilized by autoclaving

3. Preparation of seed agar

80 ml sterile molten cooled nutrient agar medium was added with suspension of *Staphylococcus aureus* & plates were poured

4. Solidification of medium

Plates were allowed to solidify

5. Preparation of wells

Wells of approximately 10 mm diameter were cut using the sterile cork borer in solidified see agar

6. Addition of honey dilutions

Each dilution of lemon was added in a separate well and proper labelling was done. In one well sterile potable water was added and it was labelled as control

10. All plates were kept in refrigerator at 4° c for 15 mints

11. After that plates were incubated at 37°c for 24 hrs.

12. After incubation plates were examined for inhibition zone around each well

Similar procedure was followed for all other pathogens

- *Salmonelly typhi*
- *Escherichia coli*
- *Proteus spp.*
- *Proteus mirabilis*



- *Pseudomonas aeruginosa*
- *Klebsiella pneumoniae*

## 2.5 Formation of health drink

The antimicrobial activity of mixture of lemon juice and honey was studied

Table 3 Dilutions of lemon juice & honey mixture

<b>Sr. No.</b>	<b>Dilution Lemon (%)</b>	<b>Dilution of Honey (%)</b>	<b>Mixture of Honey &amp; Lemon Juice (ml)</b>
1	1	1	1:1
2	10	10	1:1
3	20	20	1:1
4	30	30	1:1
5	40	40	1:1
6	50	50	1:1
7	60	60	1:1
8	70	70	1:1
9	80	80	1:1
10	90	90	1:1
11	100	100	1:1



### 2.5.1 *Staphylococcus aureus*

To study antibacterial activity of mixture of lemon juice and honey against *Staphylococcus aureus*

1. Preparation of suspension of *Staphylococcus aureus*

A loop full of freshly grown *Staphylococcus aureus* was added in to sterile saline to prepare thick suspension of organism

2. Preparation of nutrient agar

80ml nutrient agar was prepared & sterilized by autoclaving

3. Preparation of seed agar

80 ml sterile molten cooled nutrient agar medium was added with suspension of *Staphylococcus aureus* & plates were poured

4. Solidification of medium

Plates were allowed to solidify

5. Preparation of wells

Wells of approximately 10 mm diameter were cut using the sterile cork borer.

6. Addition of mixture of lemon juice and honey

Each dilution of mixture of lemon juice and honey was added in a separate well and proper labelling was done. In one well sterile potable water was added and it was labelled as control

7. All plates were kept in refrigerator at 4° c for 15 mints

8. After that plates were incubated at 37°c for 24 hrs.

9. After incubation plates were examined for inhibition zone around each well



Similar procedure was followed for all other pathogens

- *Salmonella typhi*
- *Escherichia coli*
- *Proteus spp.*
- *Proteus mirabilis*
- *Pseudomonas aeruginosa*
- *Klebsiella pneumoniae*



### 3.0 Result and Discussion

#### 3.1 Lemon

The result showed that lemon juice is effective against *Escherichia coli*, *Staphylococcus aureus*, *Proteus spp.*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Salmonella typhi*

##### 3.1.1 *Staphylococcus aureus*

Table 4 Effect of lemon on *Staphylococcus aureus*

Dilutions of lemon	1%	10%	20%	30%	40%	50%
Zone of inhibition (mm)	0.6	1.3	1.9	1.4	1.7	1.6

Dilutions of lemon	60%	70%	80%	90%	100%
Zone of inhibition (mm)	1.2	2.1	2.2	2.3	2.3

From table 4 it is clear that as concentration of lemon juice increases zone of inhibition also increases. The higher zone diameter was observed at 90% and 100% and lower zone diameter was observed at 1% concentration of lemon





Figure -1 Effect of lemon juice on *Staphylococcus aureus*.

### 3.1.2 *Escherichia coli*

Table 5 Effect of lemon on *Escherichia coli*

Dilutions of lemon	1%	10%	20%	30%	40%	50%
Zone of inhibition (mm)	-	-	-	1.4	1	2



Dilutions of lemon	60%	70%	80%	90%	100%
Zone of inhibition (mm)	2.4	2.5	2	2.4	2.5

From table 5 it is clear that as concentration of lemon juice increases zone of inhibition also increases. The higher zone diameter was observed at 70% and 100% and lower zone diameter was observed at 40% concentration of lemon. No inhibition was seen at concentrations from 1% to 30%.



Figure - 2 Effect of lemon juice on *E. coli*.

### 3.1.3 *Proteus mirabilis*

Table 6 Effect of lemon on *Proteus mirabilis*

Dilutions of lemon	1%	10%	20%	30%	40%	50%
Zone of inhibition (mm)	-	1	1.4	1.5	1.5	1.9



Dilutions of lemon	60%	70%	80%	90%	100%
Zone of inhibition (mm)	2	2	2.1	2.2	2.3

From table 6 it is clear that as concentration of lemon juice increases zone of inhibition also increases. The higher zone diameter was observed at 100% and lower zone diameter was observed at 10% concentration of lemon. No inhibition was seen at concentrations from 1% concentration.

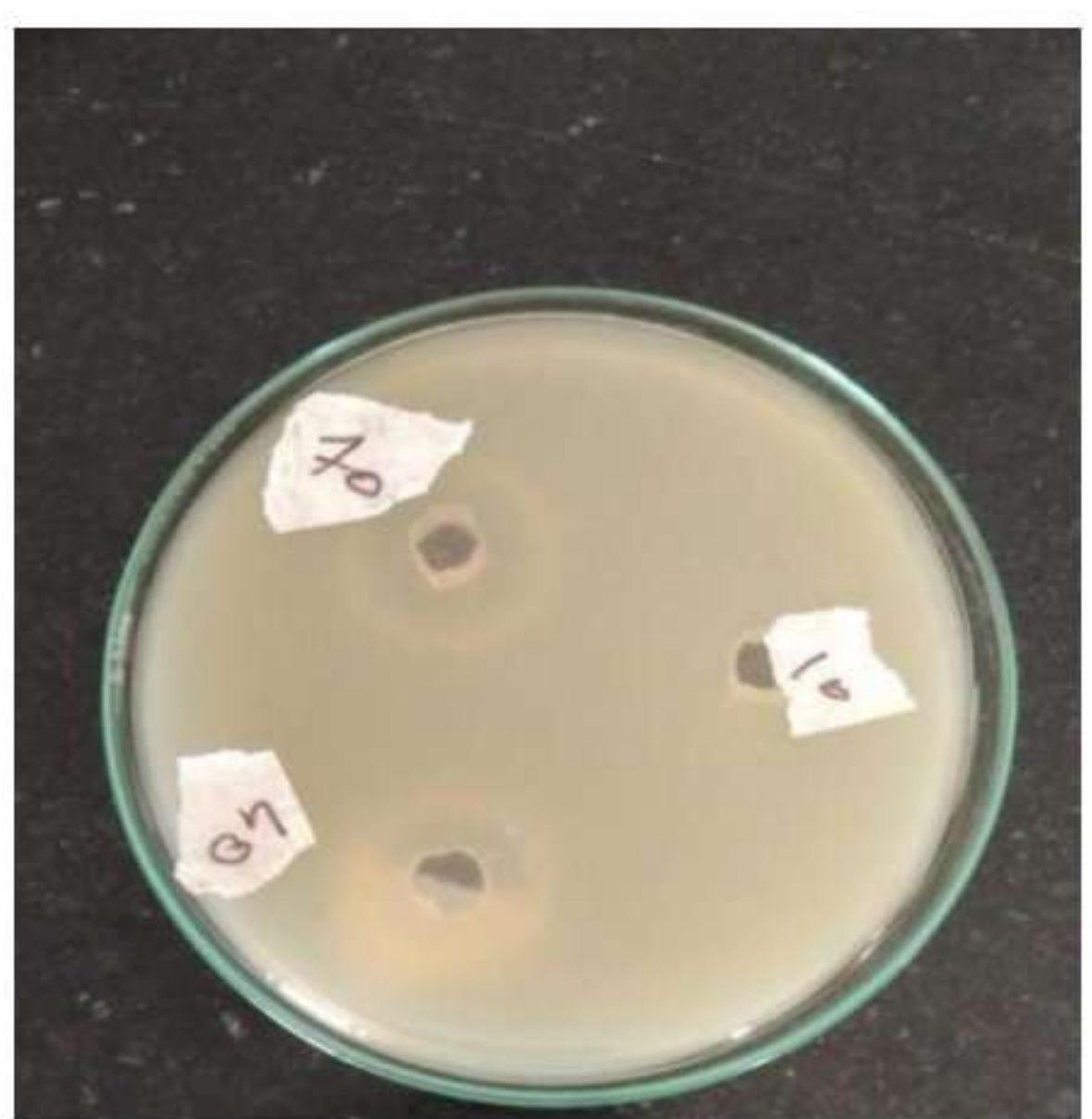
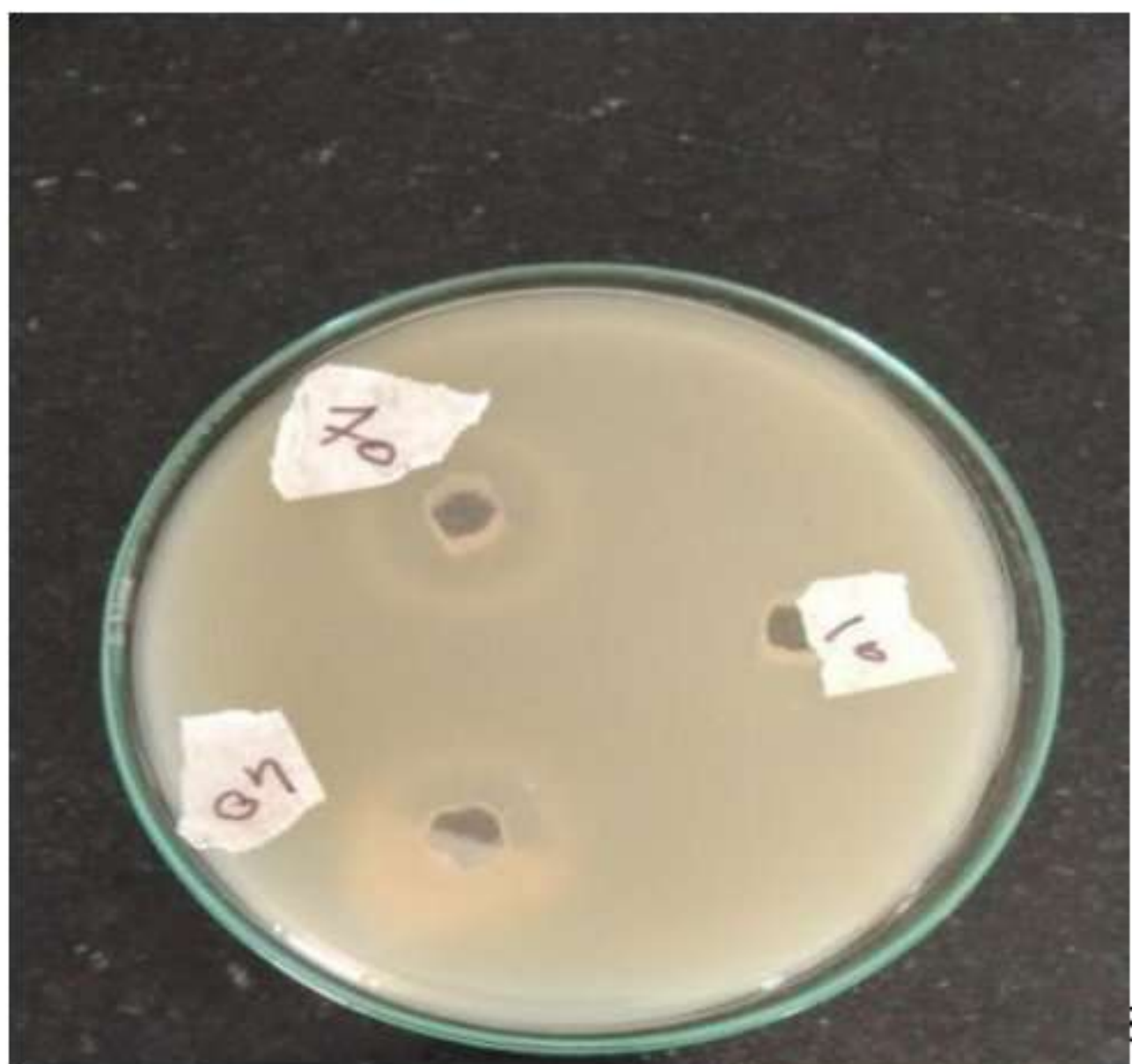






Figure - 3 Effect of lemon juice on *Proteus mirabilis*

### 3.1.4 *Pseudomonas areuginosa*

Table-7 Effect of lemon on *Pseudomonas areuginosa*

Dilution	1%	10%	20%	30%	40%	50%
Zone of Inhibition (mm)	-	-	1.9	1.3	1.9	1.9

Dilution	60%	70%	80%	90%	100%
Zone of Inhibition (mm)	2	2.1	-	2.3	2. Pure

From table 7, it is clear that as concentration of sample increases zone of inhibition also increase. The higher zone diameter was observed at 90% and lower zone diameter was observed at 30 %. No inhibition zone observes at concentration from 1% concentration of sample.



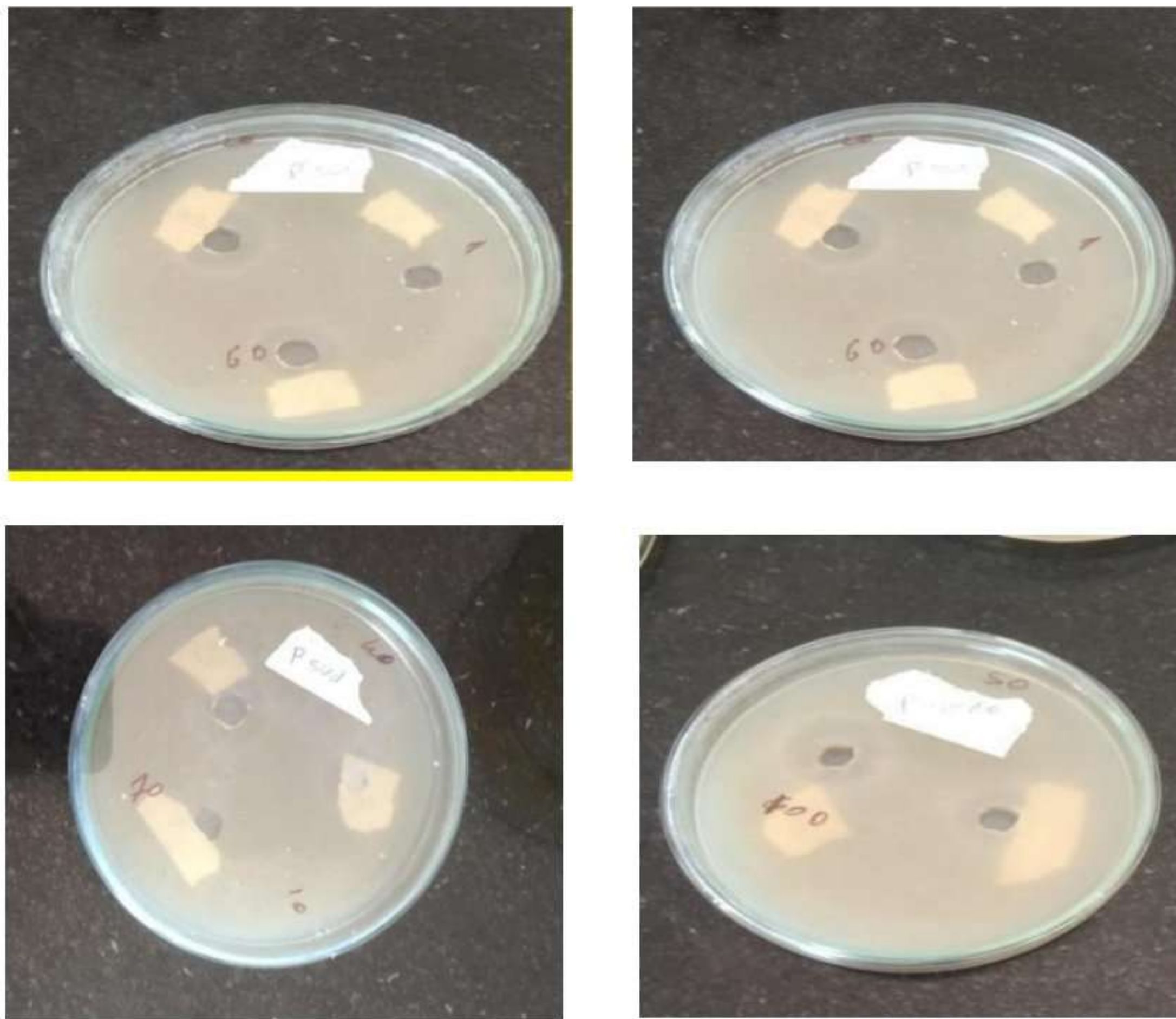


Figure - 4 The Inhibition Zone of C Juice on *Pseudomonas*

### 3.1.5 *Proteus*

Table 8 Effect of lemon on *Proteus*

Dilution	1%	10%	20%	30%	40%	50%
Zone of Inhibition (mm)	-	-	1.3	1.2	1.8	1.4

Dilution	60%	70%	80%	90%	100%
Zone of Inhibition (mm)	1.6	1.7	1.9	2.3	1.6. Pure



From above table 8, it is clear that as concentration of sample increases zone of inhibition also increase. The higher zone diameter was observed at 90% and lower zone diameter was observed at 30 %.



*Figure-5 The Inhibition Zone of C Juice on Proteus*



### 3.1.6 Salmonella

Table - 9 Effect of lemon on *Salmonella*

Dilution	1%	10%	20%	30%	40%	50%
Zone of Inhibition (mm)	-	-	0.6	1.4	1.2	1.6

Dilution	60%	70%	80%	90%	100%
Zone of Inhibition (mm)	1.7	1.9	1.9	2.2	2.3. Pure

From table 9, it is clear that as concentration of sample increases zone of inhibition also increase. The higher zone diameter was observed at 100% and lower zone diameter was observed at 20 % concentration of sample.



Figure - 6 The Inhibition Zone of C Juice on *Salmonella*



### 3.2 Honey

#### 3.2.1 *Staphylococcus Aureus*

Table -10 Effect of Honey on *Staphylococcus Aureus*

Dilution	1%	10%	20%	30%	40%	50%
Zone of Inhibition (mm)	1.5	2	2.3	2.0	2.3	2.6

Dilution	60%	70%	80%	90%	100%
Zone of Inhibition (mm)	2.8	2.7	3.1	3	4 Pure

From table 10, it is clear that as concentration of sample increases zone of inhibition also increase. The higher zone diameter was observed at 100% and lower zone diameter was observed at 1% concentration of sample.

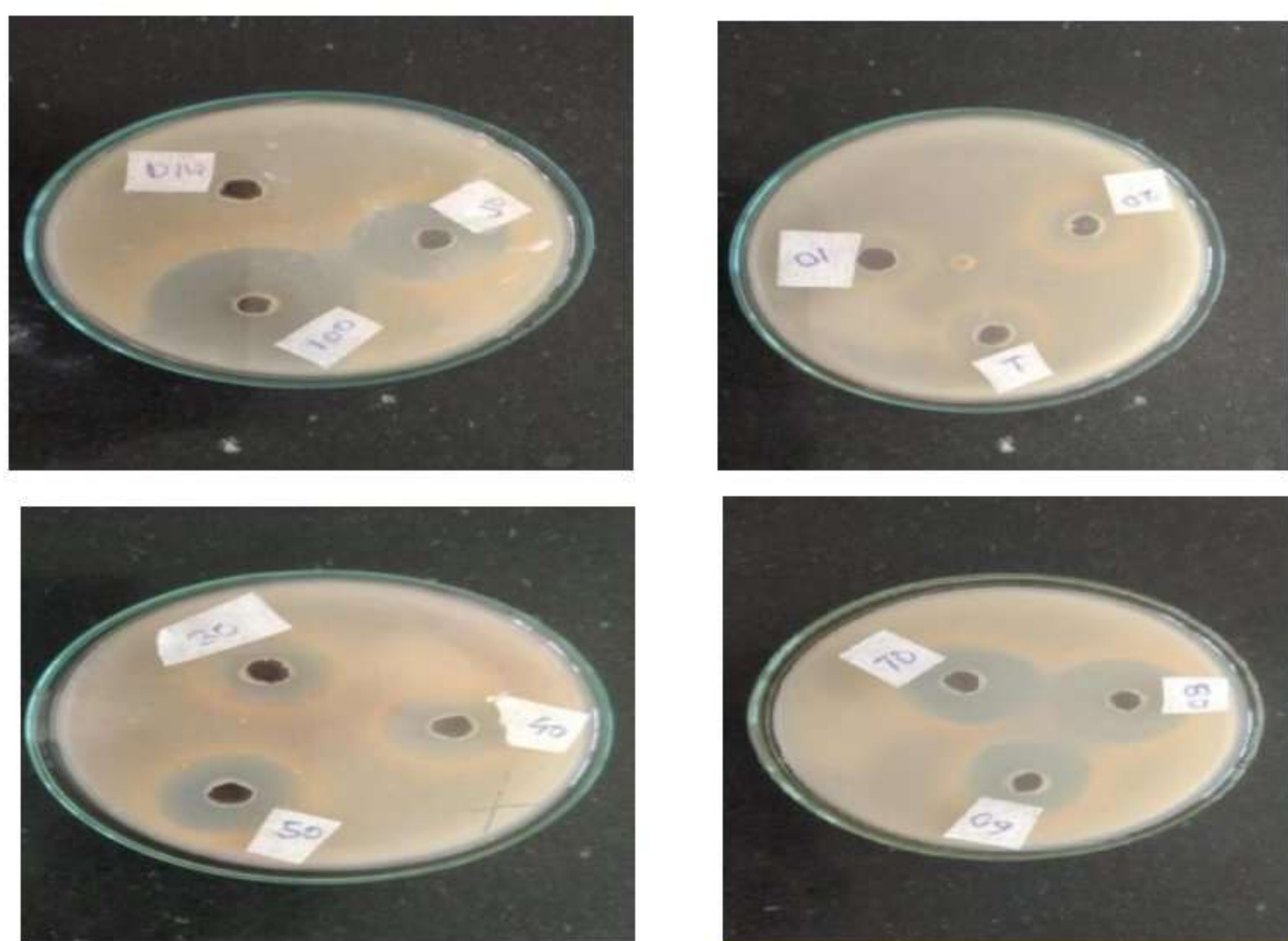


Figure - 7 The Inhibition Zone of C Juice on *Staphylococcus Aureus*



### 3.2.2 *Escherichia Coli*

Table – 11 Effect of Honey on *Escherichia coli*

Dilution	1%	10%	20%	30%	40%	50%
Zone of Inhibition (mm)	-	1.9	2.7	2.6	3.0	3.0

Dilution	60%	70%	80%	90%	100%
Zone of Inhibition (mm)	3.1	3.5	3.6	3.1	3.8 Pure

From table 11, it is clear that as concentration of sample increases zone of inhibition also increase. The higher zone diameter was observed at 100% and lower zone diameter was observed at 10% concentration of sample. There is no observer inhibitory zone of 1%.

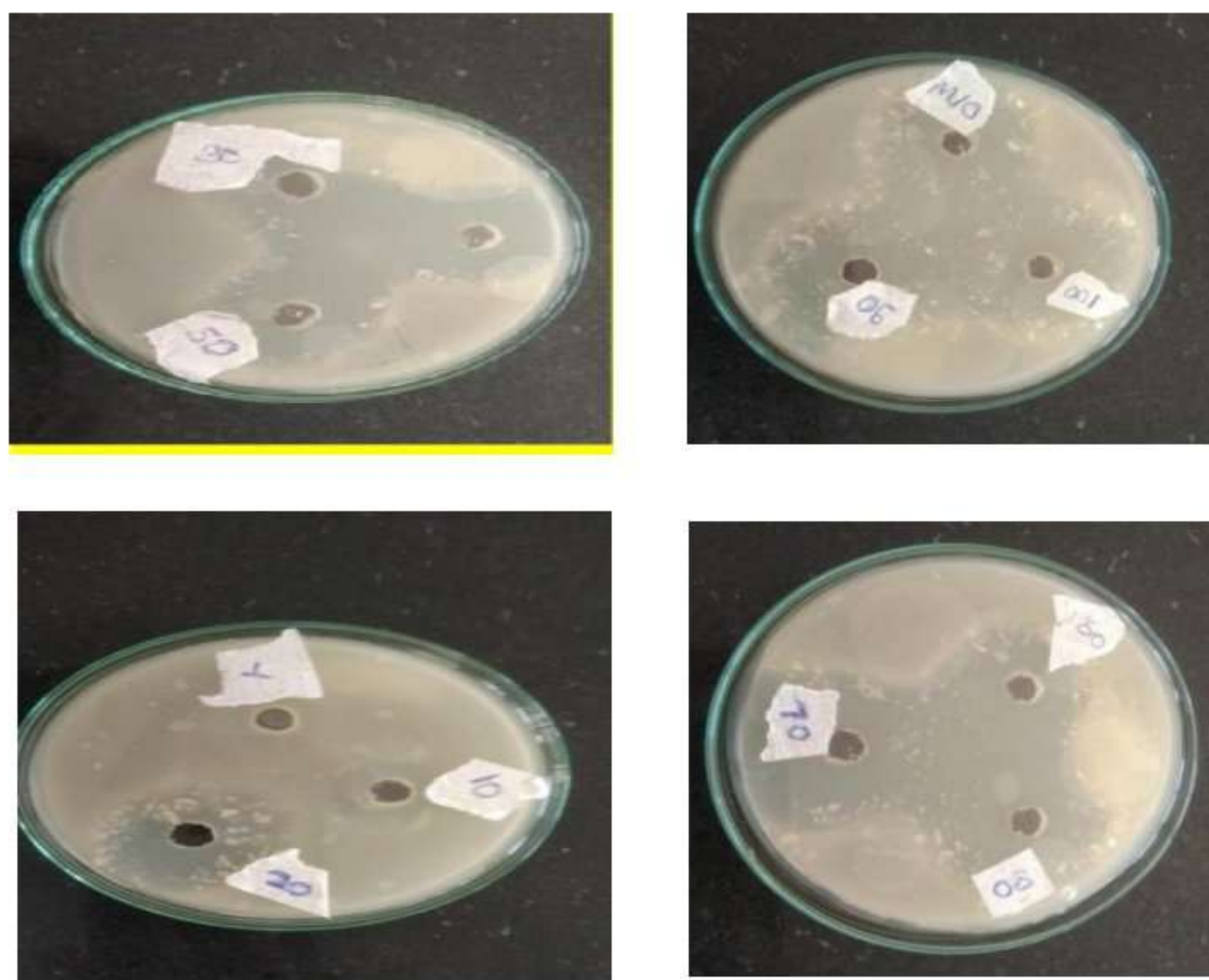


Figure - 8 The Inhibition Zone of C Juice on *Escherichia coli*



That Result Shows this Honey do not effective against *Escheria Coli*, *Proteus*, *Pseudomonas* and *Salmonella*. There is no inhibition zone observe.

### 3.3 Combination of honey and lemon

The mixture of lemon and honey of different proportions of healthy drink were tested against microbial isolates cause infection off *Staphylococcus Aryas*, *Salmonelly*, *Pseudomonas*, *Klebsiella*.

Table -12

Sr. No	Dilution Lemon and Honey	S. aureus	Pseudomonas	Salmonelly	Klebsiella
1	1	1	-	-	-
2	10	1	-	-	-
3	20	2	-	-	0.9
4	30	1.2	1.1	1.2	1.1
5	40	1.3	-	-	1.3
6	50	1.6	1.1	1.3	1.2
7	60	1.3	1.4	1.3	1.4
8	70	1.5	1.5	1.5	1.7
9	80	1.6	1.5	1.2	1.4
10	90	1.6	1.9	1.5	1.6
11	100	1.5	2	1.5	1.5



### 3.3.1 *Staphylococcus aureus*

**Table -13**

Dilution	1%	10%	20%	30%	40%	50%
Zone of Inhibition (mm)	1	1	2	1.2	1.3	1.6

Dilution	60%	70%	80%	90%	100%
Zone of Inhibition (mm)	1.3	1.5	1.6	1.6	1.5 Pure

From table 11, it is clear that as concentration of sample increases zone of inhibition also increases. The higher zone diameter was observed at 80%, 90% and 100% and lower zone diameter was observed at 1% and 10% concentration of sample.



**Figure -7** The Inhibition Zone of C. Juice on *Staphylococcus*



### 3.3.2 Salmonelly

Table-14

Dilution	1%	10%	20%	30%	40%	50%
Zone of Inhibition (mm)	-	-	-	1.2	-	1.3

Dilution	60%	70%	80%	90%	100%
Zone of Inhibition (mm)	1.3	1.5	1.2	1.5	1.5 Pure

From table 14 it is clear that as concentration of sample increases, zone of inhibition also increases the higher zone of diameter was observed at 90%, 100% and 70 % with lower zone diameter was observed at 30% concentration of sample. There is no observer inhibitory zone of 1%, 10% and 20%.



Figure - 8 The Inhibition Zone of C. Juice on *Salmonella*



### 3.3.3 *Pseudomonas*

Table -15

Dilution	1%	10%	20%	30%	40%	50%
Zone of Inhibition (mm)	-	-	-	1.1	-	1.1

Dilution	60%	70%	80%	90%	100%
Zone of Inhibition (mm)	1.4	1.5	1.5	1.9	2 Pure

From table 11, it is clear that as concentration of sample increases zone of inhibition also increases. The higher zone diameter was observed at 80%, 90% and 100% and lower zone diameter was observed at 1% and 10% concentration of sample.



Figure – 9 The Inhibition Zone of C. Juice on *Pseudomonas*



### 3.3.4 *Klebsiella Pneumoniae*

Table -16

Dilution	1%	10%	20%	30%	40%	50%
Zone of Inhibition (mm)	-	-	0.9	1.1	1.3	1.2

Dilution	60%	70%	80%	90%	100%
Zone of Inhibition (mm)	1.4	1.7	1.4	1.6	1.5 Pure

From table 11, it is clear that as concentration of sample increases zone of inhibition also increase. The higher zone diameter was observed at 100% and with lower zone diameter was observed at 20% concentration of sample. There is no observer inhibitory zone of 1% and 10%.



Figure - 10 The Inhibition Zone of C. Juice on *Klebsiella Pneumoniae*



### 3.4.1 Organoleptic Testing

Sr. No	Name Of Student (Male & Female)	Dilution	Test	Viscosity
		1:1+2 water		
1	Ankita	L+H+W	Sour	Highly Viscous
2	Namrata	L+H+W	Sour	Highly Viscous
<b>1:1+3ml Water</b>				
1	Rachana	L+H+W	Partially Sour	Less Viscous
2	Snehal	L+H+W	Partially Sour	Less Viscous
3	Shivam	L+H+W	Partially Sour	Less Viscous
<b>1:1+4ml Water</b>				
8	Ankita	L+H+W	Slightly Sour	Liquid form
9	Namrata	L+H+W	Slightly Sour	Liquid form
10	Harshada	L+H+W	Slightly Sour	Liquid form
11	Suhasi	L+H+W	Slightly Sour	Liquid form
12	Snehal	L+H+W	Slightly Sour	Liquid form
13	Vishal	L+H+W	Slightly Sour	Liquid form



## 4.0 Summary and Conclusion

The use of different concentration of C. Juice and honey has effective antimicrobial activities against *Staphylococcus aureus*, *Proteus Spp.*, *Proteus Mirabilis*, *Salmonella*, *Escherichia coli*, *Pseudomonas aeruginosa*. The results indicated that the C. Juice and honey has antimicrobial activities of this combinations has such a level that it would be useful and it is possible that this combination may have clinically applicable for treatments of certain infections.

One of the most important sources of medicines are plants. It is well documented from ancient times till date all over the world, medicinal plants are stimulating the ability of human health to handle with the unpleasant and difficult circumstances. A millennium development goal (MDGs) has one of the fundamental goals which is the quest to combat the incidence of diseases such as kidney stone, respiratory disorder, cancer blood sugar balance and cardiovascular diseases. The potential sources of drugs are secondary metabolites are rich in medicinal plants. As therapeutic agents, the interest in the use of plant extracts is increasing. A "Citrus Fruit" lemon belongs to family Rutaceae which is used in pharmacological potential as solution. One of the most popular fruits is lemon in all over the world. From the literature investigation, it is stated that a potential source of anti-diabetic, anticancer, antimicrobial and anti-inflammatory activity is lemon.