COMPARISON BETWEEN THE ACTIVITIES OF EXTRACTS OF *ALLIUM SATIVUM* (GARLIC) AND *ALLIUM SCHENOPRASUM* (SNOW MOUNTAIN GARLIC) AGAINST BACTERIAL SPECIES

Dissertation submitted in partial fulfilment of requirements for the

Degree of

BACHELOR OF TECHNOLOGY

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BY

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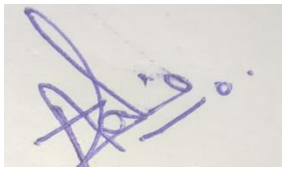
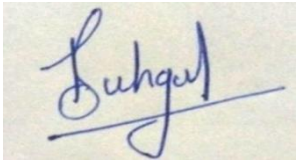
DECLARATION BY THE SCHOLARS

We hereby declare that the work reported in the B.tech entitled **"Comparison between activities of extracts of *Allium sativum* and *Allium schoenoprasum* against bacterial strains"** submitted at **Jaypee University of Information Technology, Waknaghat –India** is an authentic report of work done by (Aashina Puri -161823) and (Tanvi Sehgal-161830) under the supervision of Dr**. Rahul Shrivastava (**Associate Professor) Department of Biotechnology and Bioinformatics. We have not submitted the work elsewhere for any degree or diploma.





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Date: 30.06.2020

SUPERVISOR CERTIFICATE

This is to certify that the work reported in B.tech entitled **"Comparison between activities of extracts of Allium sativum and Allium schoenoprasum against bacterial strains"** submitted at **Jaypee University of Information Technology, Waknaghat –India** by **Aashina Puri (161823)** and **Tanvi Sehgal (161830)** is a bonafide record of their original work carried out under my supervision. This work has not been submitted elsewhere for any other degree or diploma.

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We would like to express our sincere gratitude and appreciation to Mr. Baleshwar and Mrs. Mamta who gave their precious time in helping us and providing us with all the chemicals and equipments required during the project.

We bow our head before the **Almighty God** whose blessing gave us the strength to make this successful venture and we dedicate our work and achievement in his lotus feet.

Aashina Puri (161823)

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ABSTRACT

Garlic has been of tremendous importance in scientific research because of its extended medicinal properties. Various strains of micro organism are inhibited by components of crushed garlic due to its antimicrobial properties along with this garlic juices have shown their effect on strains showing extreme drug resistance and multiple drug resistance. The antimicrobial properties of garlic are constituted by various components present in it. Allicin is on such constituent who inhibit the growth of wide range bacteria both being gram positive or gram negative. The fresh garlic cloves consist of alliin when garlic cloves are crushed he enzyme allinase is released which converts alliin to allicin which is responsible for aroma of garlic.

Through our work on garlic extracts on bacterial species a little amount of activity was observed in case of *Salmonella typhi* out of other 3 bacterial strains. No activity was observed in case of Snow Mountain Garlic extract on any bacterial strains.

Keywords: Antimicrobial, Garlic, Snow Mountain Garlic, *Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Mycobacterium smegmatis*

CHAPTER 1

INTRODUCTION

Medicinal plants grow all over the world but the medicinal plants found in the Himalayan region have lot of different significances. Himalayas have always been a rich heritage of knowledge on medicinal plants and harbors a good number of medical plants like *Picrorhiza kurroa, Curcuma longa, Allium sativum, Azardirachta indica.* Himalayan medicinal plants are very specific to a region.

The ability of medicinal plants to produce phytochemicals is of great beneficence. Their medicinal properties like antioxidant, anti-inflammatory, antifungal, antiviral and antibacterial have been reported in various journals, research papers and publications. These properties of medicinal plants and their ability to cause zero or very less side effects have drawn consciousness of lot of companies. Nowadays, lot of businessmen is investing their money in the pharmaceutical companies that are dealing with the Himalayan Medicinal Plants in order to study their pharmacological properties. Rather than using the whole plants, companies are preparing modern medicines that only use the active ingredients of plants.

Diseases are the major cause of death in this world and microbial resistance to antibiotics poses a major challenge to the research sector. Various medicinal plants tend to inhibit the growth of different microorganisms that have shown Multiple Drug Resistance and Extreme Drug Resistance.

Salmonella typhi, Staphylococcus aureus, Bacillus subtilis and *Mycobacterium* species *are major* microbes for various food born diseases, stomach related infection and skin infections. To compact the various effects of these microbes various medicinal plants extracts are tested and one such plant employed is garlic whose various extracts have shown major effect on the microbes in terms of their growth. On the counter part Snow Mountain Garlic a subspecies of Garlic is less studied and researched.

The present study aims to compare the activities of extract prepared from Garlic and Snow Mountain Garlic (a subspecies of garlic found in Kashmir belt of India) against various microbes

particularly *Bacillus subtilis, Staphylococcus aureus Salmonella typhi* and *Mycobacterium* The extracts being studied are water extracts and ethanolic extracts of Garlic and Snow Mountain Garlic. The activity of these extracts was checked against microbes by determining the zones of inhibition through agar diffusion method and the concentration was determined through Minimum Inhibitory Concentration (MIC). The end result of the study would provide us with relevant results on basis of which the two can be compared and hence, can be further employed for research purposes.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Garlic:

Garlic in recent arena has been one of the most important medicinal plants. Garlic juices exhibit antibacterial, antifungal and antiviral properties despite has components which boost immune system. Garlic juices have shown potent effect on certain strains of microbes which exhibit XDR (Extreme Drug Resistance) and MDR (Multiple Drug Resistance) against all possible drugs. Example various strains of *Mycobacterium.* [33]

2.11 Garlic Chemistry:

In 1884 **Wertheim** through steam distillation isolated the garlic oil from garlic cloves that had characteristic pungent smell. [17] These garlic juices consisted of the hydrocarbon chains which he named allyl. Later in 1944 **Cavallito** and **Bailey** isolate diallyl disulfide from these garlic juices other major component allicin was also isolated from garlic juice by them. These components impart various antimicrobial properties to garlic. [33]

Steam Distillation (100˚C) = Diallyl Disulfide
Ethanol and Water (25˚C) = Allicin
Ethanol (<0˚C) =Alliin

Figure2.1 Different compounds of sulphur extracted from garlic juice under suitable conditions) [8]

2.2 *Allium schoenoprasum:* the smallest member of *Allium* genus and is commonly known as chives. This species of garlic particularly inhibit high altitude region of 5000-8000 feet. In India this type of garlic is present in Kashmir hence is also known as **Kashmiri garlic** or **snow mountain garlic**. [16]

Nutrition Chart of Garlic			
Energy	149 Kcal	Electrolytes	
Carbohydrates	33.06 g	Sodium	153 mg
Protein	6.36 g	Potassium	401 mg
Total Fat	0.5 g	Minerals	
Cholesterol	0 mg	Calcium	181 mg
Dietary Fiber	2.1 g	Copper	0.299 mg
Vitamins		Iron	1.70 mg
Folates	3 µg	Magnesium	25 mg
Niacin	0.700 mg	Manganese	1.672 mg
Pantothenic acid	0.596 mg	Phosphorus	153 mg
Pyridoxine	1.235 mg	Selenium	14.2 µg
Riboflavin	0.110 mg	Zinc	1.160 mg
Thiamin	0.200 mg	Phyto-nutrients	
Vitamin A	9 IU	Carotene-ß	5 µg
Vitamin C	31.2 mg	Crypto-xanthin-ß	0 µg
Vitamin E	0.08 mg	Lutein-zeaxanthin	16 µg
Vitamin K	1.7 µg		

Figure2.2 Nutrition properties of Allium sativum (normal garlic) [33]

Minerals	Value per gram
Calcium	92 mg
Iron	1.60 mg
Magnesium	42 mg
Phosphorus	58 mg
Potassium	296 mg
Sodium	3 mg
Zinc	0.56 mg
Copper	0.157 mg
Manganese	0.373 mg
Selenium	0.9 µg

Vitamins	Value per gram
Vitamin C	58.1 mg
Thiamin	0.078 mg
Riboflavin	0.115 mg
Niacin	0.647 mg
Pantothenic acid	0.324 mg
Vitamin B6	0.138 mg
Total Folate	105 µg
B-carotene	2612 µg
Vitamin A	4353 IU
Vitamin E	0.21 mg
Vitamin K	212.7 µg

Lipids	Value per gram
Total saturated fatty acids	0.146 g
Total monounsaturated fatty acids	0.095 g
Total polyunsaturated fatty acids	0.267 g
Phytosterols	9 mg

Amino acids	Value per gram
Tryptophan	0.037 g
Threonine	0.128 g
Isoleucine	0.139 g
Leucine	0.195 g
Lysine	0.163 g
Methionine	0.036 g
Phenylalanine	0.105 g
Tyrosine	0.095 g
Valine	0.145 g
Arginine	0.237 g
Histidine	0.057 g
Aspartic acid	0.303 g
Glutamic acid	0.677 g
Glycine	0.162 g
Proline	0.216 g
Serine	0.148 g

Figure2.3 Nutrition Chart of *Allium schoenoprasum* (Snow Mountain Garlic) [34]

2.3 Antibacterial properties of garlic:

Various ethanolic and water extracts of garlic have effectively shown its inhibitory results on both strains of bacteria gram negative and gram positive along with certain acid fast microbes. [12] Various AST and MIC data has been generated for different bacterial organisms including *Salmonella Staphylococcus, Bacillus* and *Mycobacterium* species. The antimicrobial properties of garlic are attributed to allicin component of garlic. [4]

2.31 Allicin: A thioester of sulfonic acid responsible for fresh aroma of garlic and providing garlic its respective antimicrobial properties. [8] Allicin is present in cells of garlic and is oily and slightly yellow in colour. It is released from garlic cells only when cloves of garlic are crushed or cooked. [33]

2.32 Structure: The functional group present in allicin is thiosulfinate **R-S (O)-S-R**. As mentioned earlier when garlic tissue is damaged the enzyme allinase acts on alliin component of the garlic cells and release allinase. [8]

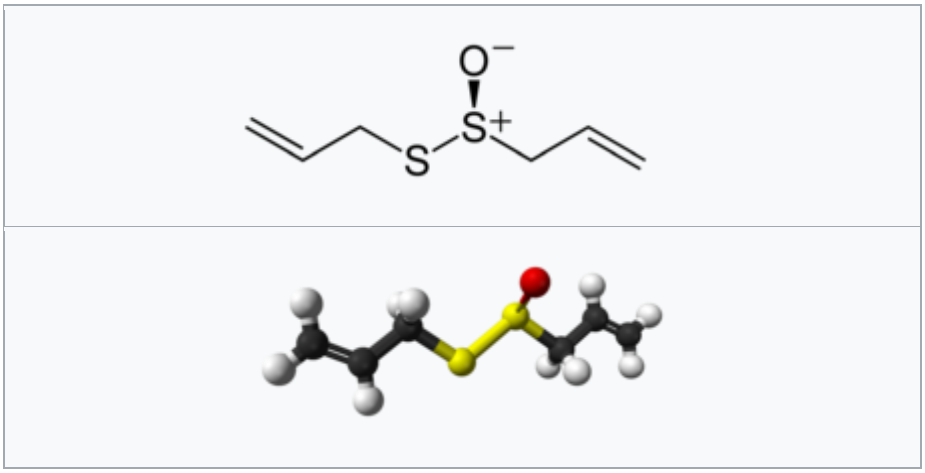


Figure2.4 Structure of Allicin [8]

The enzyme allinase is highly unstable at pH 3 this is the reason there is no production of allicin in one's body after consumption of fresh garlic. Furthermore allicin produced is highly degradable and is degraded within 16hrs of extraction if not stored in cold conditions.

Properties of Allicin	
Chemical formula	$C_6H_{10}OS_2$
Molar Mass	162.26 g·mol^{-1}
Appearance	Colourless liquid
Density	1.112 g cm^{-3}
Melting Point	< 25 °C (77 °F; 298 K)
Boiling Point	Decomposes

Figure2.5 Allicin Properties [8]

2.4 Research carried on garlic:

The potential of allicin compound of garlic is tested to treat infections caused by **Multidrug resistance species** as well as infections caused by viruses and fungus. [18] Various articles reported that garlic extract work well against common cold virus. [18]

Significant amount of research articles are present which exhibit the effect of garlic extract on various food borne pathogens hence treating food poisoning and other skin infections caused by *Staphylococcus aureus* [12]. The most significant research has been the activity of garlic extract against various strains of *Mycobacterium*. Be it *Mycobacterium spegmatis, Mycobacterium fortuitum* or *Mycobacterium tuberculosis* the deadliest of all, garlic extract have shown potent positive results in treating infections caused by these microbes. [4]

2.5 Work of garlic extracts on certain pathogenic strains:

2.51 *Salmonella typhi:*

Salmonella is gram negative rod shaped bacterium belonging to family of *Enterobacteriaceae. Salmonella typhi* is for responsible for causing the deadly **typhoid fever**. [15] Typhoid fever nearly affects 21.6 million people around globe every year. 62% of these cases are prevalent in Asia with 1.06 million cases alone in India with 346 deaths every year. [10]

Infections caused by *Salmonella*

There are over 2000 different types of Salmonella bacteria. Various infections are caused by *Salmonella* bacteria. Mostly, this bacteria causes gastroenteritis that is the inflammation of the intestine which leads to diarrhea and sometimes, it causes typhoid fever and paratyphoid fever, which are more severe forms of infection. [15]

Salmonella can infect humans through various means like:

- Eating and drinking contaminated food and water
- Touching infected animals or their feces.

Figure 2.6 Thyroid fever

Treatment

Various antibodies such as quininole, macrolide, and third generation cephalosporin's are widely used to treat infections caused by *Salmonella typhi* but recent trends showed increased resistance of concerned microbe against the antibiotics employed. To compact this problem various extracts of medicinal plants are employed along with antibiotics to control the growth of concerned microbe. One such extract used is Garlic extract.

Work of *Allium extract* on *Salmonella typhi:*

Various crude extracts of garlic such as ethanolic extract, methanolic extract and n-hexane extracts show varying activity on *Salmonella.* Even normal water extract of garlic have shown bacteristatic effects on concerned microbe. To be more precise methanolic extracts have better activity as per the literature. Snow Mountain Garlic on other hand is not extensively researched on the extracts activity. [1]

2.52 *Staphylococcus aureus:*

It is a gram positive rod shaped bacteria which forms the microbiota of our body. It is present on top surface of the skin, gastrointestinal tract and mucus membrane. [27] Despite of forming microbiota of our body it can act as **opportunistic microbe** causing wide range variety of infection starting from skin infections to food poisoning. [27]

Infections caused by *Staphylococcus aureus*

The infections caused by Staphylococcus can range from mild skin infections to life threatening sepsis and pneumonia. Skin infections can lead to boils, pimples, impetigo (crusting of skin), cellulites (inflammation of the deeper layers of the skin). [24] In breastfeeding women, mastitis that is inflammation of the breast can occur that can also contaminate the mother's milk.

Sepsis occurs when the bacteria enters into the blood stream. It is called life threatening because Staphylococcal sepsis leads to shock, circulatory collapse and death in several cases. Sometimes,

it also causes choriomeningitis and neonatal sepsis in pregnancy which is also life threatening to the developing foetus. [24]

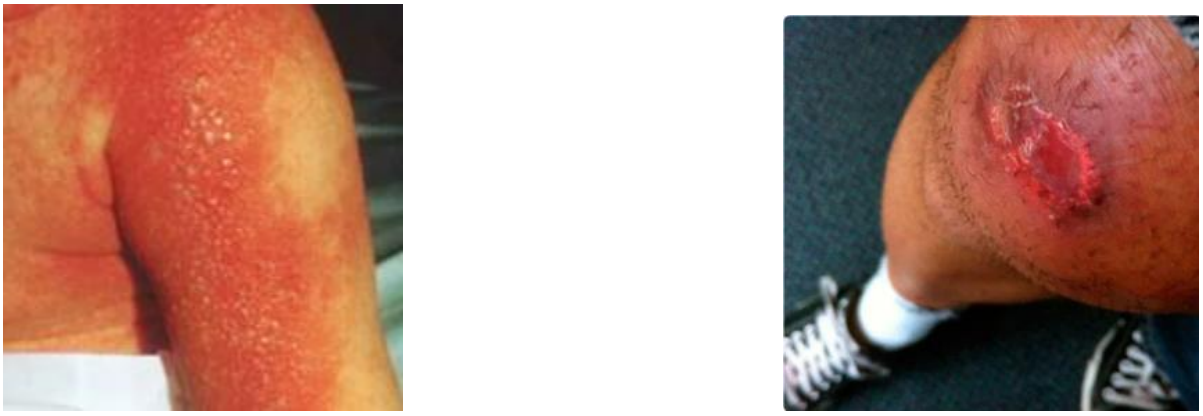


Figure 2.7 Skin infections caused by *Staphylococcus aureus*

Treatment

Minor skin infections can be treated with a simple antibiotic ointment whereas for other forms of infections, various antibiotics are used to treat the infection like nafcillin, cefuroxime, vancomycin, oxacillin, rifampin and clindamycin. [24]

Work of Allium extracts on *Staphylococcus aureus:* Again garlic extracts have shown both bacteristatic and bactericidal effect on the growth of *Staphylococcus aureus.* The prepared garlic extracts have direct effect on skin infections such as pimples, pus etc. Garlic has proven to show its effect to control food poisoning cases. Again Snow Mountain Garlic extracts activity has not been studied [12] [23]

2.53 *Bacillus subtilis:*

Also known as grass bacillus or hay bacillus is a gram positive bacterium. This bacteria is common in soil and is also found in gastrointestinal tract of human begins and ruminants. This bacterium is rod shaped and develops a tough endospore covering outside its cell membrane making it to tolerate tough environment conditions. [36]

Role in diseases: *Bacillus* species are not considered pathogenic but have role in causing bacteremia, endocarditis, meningitis and infections of wounds and rare infection in ear, eyes etc infections of gastrointestinal tract, respiratory and urinary tract. [30]

Treatment

Bacillus infections can be treated by using various antibiotics such as clindamycin, vancomycin, kanamycin and erythromycin. [36]

Work of Allium extracts on *Bacillus subtilis:* Garlic extracts have shown good effect on activity of *Bacillus anthracis* and similarly it exhibit bactericidal impacts on growth of *Bacillus subtilis*. The activity of Snow Mountain Garlic has not been tested so far on this species.

2.54 *Mycobacterium smegmatis:*

Belonging to phylum Actinobacteria and genus Mycobacterium it is acid fast bacteria. It is a bacillus shaped bacteria with 3.0 to 5.0 µm in length. [28]

Virulence: *M.smegmatis* is generally a non pathogenic bacterium and is not responsible in causing any serious condition or diseases in humans as compared to other counterparts of Mycobacterium. But has its **important role in research** and is commonly used in reference for work on other Mycobacterium species **as it is a fast grower and is non-pathogenic in nature**. Therefore it is used as a **model organism** to study other Mycobacterial species. [30]

Infections caused by *Mycobacterium smegmatis*

There are very less number of cases M. *smegmatis* infection in humans have been reported. The infections like soft-tissue or skin infections following cardiac surgery or traumatic injury in immunocompetent individuals have been seen. [7]

Prosthetic knee joint infection, granuloma in soft tissues, chronic cellulites with fistula formation, catheter infections and pulmonary infections are common. [18]

Treatment

These rare infections can be treated using antibiotics like unopened, ciprofloxacin, amikacin, doxycycline, ethambutol and sulfamethoxazole. [28]

CHAPTER 3

MATERIALS AND METHODS:

3.1 Test Microorganisms

Cultures of *Bacillus subtilis*, *Salmonella typhi*, *Staphylococcus aureus* and *Mycobacterium smegmatis* were collected from the lab of Department of Biotechnology, Jaypee University of Information Technology and they were confirmed by growing them in their respective media. Then, they were cultured in 5ml of liquid media each.

3.2 Antibiotics

Antibiotics discs, Tetracycline, Gentamicin and Kanamycin were also taken from the lab of Department of Biotechnology, Jaypee University of Information Technology.

3.3 Garlic Bulbs

The bulbs of *Allium sativum* and *Allium schoenoprasum* were brought from the local market. *Allium sativum* and *Allium schoenoprasum* bulbs were peeled and chopped.

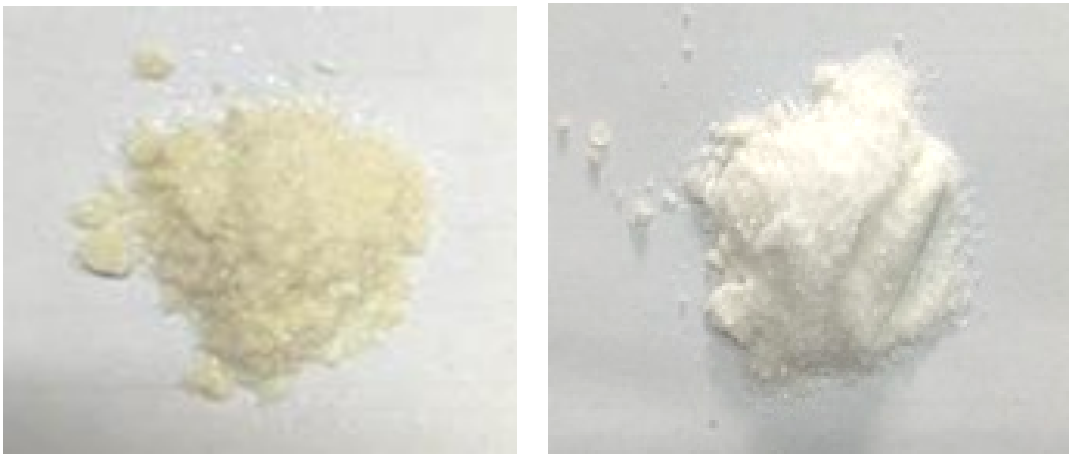
(a) **(b)**

Figure3.1 a) Chopped *Allium sativum* b) Chopped Allium schoenoprasum

3.4 Preparation of *Allium* Extract

3.41 Water Extract [36]

- 90g of both the types of bulbs were weighed and it were mixed with 200ml of water and blended in a blender for 15 min.
- With the help of muslin cloth, solid parts from both the garlic juices were removed and then, the solution was centrifuged for 30min at 4500rpm at 20℃.
- Pellet obtained was filtered out and discarded and the supernatant from both the garlic juices were transferred in round bottom flasks and stored at -80℃ for a day.
- Next day, both the samples were lyophilized and the powdered water extracts were collected.



(a) **(b)**

Figure3.2 a) Lyophilized extract of *Allium sativum* b) Lyophilized extract *Allium schoenoprasum*

3.42 Ethanol Extract [35]

- 90g of bulbs from each garlic variety were weighed and cut into small pieces.
- The bulbs were mixed with 100ml of 96% ethanol in two different flasks. Then, flasks were kept at dark for 10 days with mixing at regular intervals.
- After 10 days, the solid parts were removed from both the flasks with the help of muslin cloth.
- The solutions were transferred to the respective tarsons and centrifuged at 4500rpm for 30min at20℃.
- The pellets from tarsons were removed and the supernatant were filtered and transferred in two different round bottom flasks.
- Next day, with the help of Rota Evaporator, the ethanol from the extracts were evaporated which led to sticky normal garlic extract and sticky snow mountain garlic extract.

Figure3.3 Ethanolic extract of *Allium sativum*

Figure3.4 Ethanolic extract of *Allium schoenoprasum*

(The labelled portion)

3.5 Preparation of Media

- Flasks of Luria broth agar 150ml each were prepared and autoclaved.
- After autoclaving, the media was poured into autoclaved petriplates inside the Laminar Air Flow and kept at 37℃ for one day for contamination check.
- For ***Mycobacterium smegmatis*** **NAT** media was prepared.

3.6 Determination of Antibacterial Activity of Allium *sativum* and *Allium schoenoprasum* Extract (Water and Ethanol Extract)

1. For *Salmonella typhi*

- The stocks of **25mg/mL** of water and ethanol extracts of both types of garlic were prepared.
- Inside the Laminar Air Flow, the culture of *Salmonella typhi* was spread on previously incubated petriplates.

- With the help of sterile cork borer, 4 wells were punched on each plate and different concentrations of garlic extract were added in it.
- **The concentrations were: 18mg/mL, 20mg/mL, 21mg/mL and 22mg/mL**
- In another plate, 3 wells were punched, one for positive control in which antibiotic was placed **(antibiotic used Tetracycline**) and other two for solvents (water and ethanol).
- The plates were then, kept in incubator at 37°C for a day.
- Next day, the results were observed.

2. For *Staphylococcus aureus*

- Different concentrations of available extracts were prepared.
- The culture of *Staphylococcus aureus* was spread evenly on previously incubated plates.
- With help of sterile cork borer, 4 wells were punched on the plate and different concentrations of garlic extract were added in it.
- **The concentrations were: 40mg/mL, 60mg/mL, 80mg/mL and 100mg/mL**
- In another plate, 3wells were punched, one for positive control in which antibiotic was placed (**antibiotic used Tetracycline)** and other two for solvents (water and ethanol)
- The plates were then, kept in incubator at 37˚C for a day.
- Next day result was observed.

3. For *Bacillus subtilis*

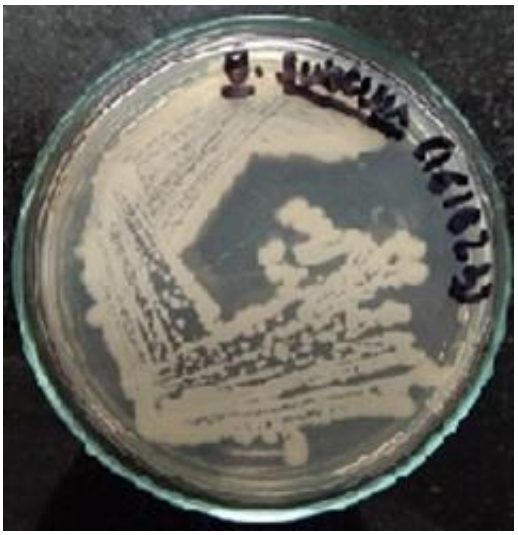
- Stocks of 50mg/ml were prepared of ethanol and water extracts for both types of garlic.
- The culture of *Bacillus subtilis* was spread evenly on previously incubated plates.
- With help of sterile cork borer, 4 wells were punched on the plate and different concentrations of garlic extract were added in it.
- **The concentrations were 1mg/mL, 5mg/mL, 10mg/mL and 40mg/mL**
- In another plate, 3wells were punched, one for positive control in which antibiotic was placed (**antibiotic used Gentamicin)** and other two for solvents (water and ethanol)
- The plates were then, kept in incubator at 37˚C for a day.
- Next day result was observed.

4. For *Mycobacterium smegmatis*

- Different concentrations of extract were prepared.
- The culture of *Mycobacterium smegmatis* was spread evenly on previously incubated plates.
- With help of sterile cork borer 4 wells were punched on the plate and different concentrations of garlic extract were added in it.
- **The concentrations were 2mg/mL, 40mg/mL, 60mg/mL and 80mg/mL**
- In another plate, 3wells were punched, one for positive control in which antibiotic was placed (**antibiotic used Kanamycin)** and other two for solvents (water and ethanol)
- The plates were then, kept in incubator at 37˚C for a day.
- Next day result was observed.

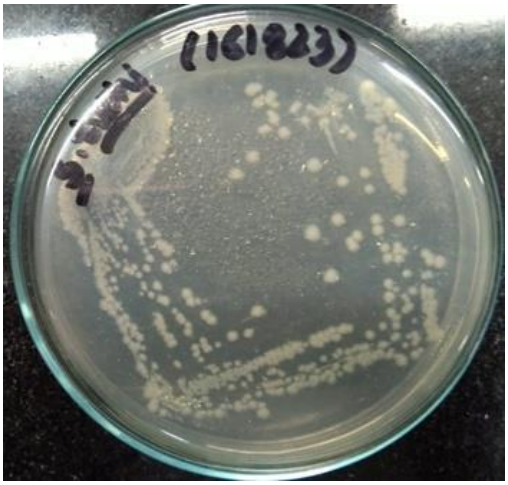
CHAPTER 4

RESULTS AND DISCUSSION



(a)

(b)



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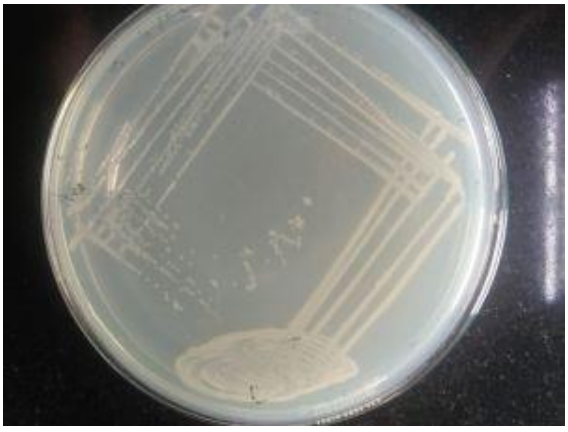

(d)

Figure 4.1 Result of streaking a) *Bacillus subtilis* b) *Salmonella typhi* c) *Streptococcus aureus* d) *Mycobacterium smegmatis*

4.2) Result for experiment done with *Salmonella typhi:*

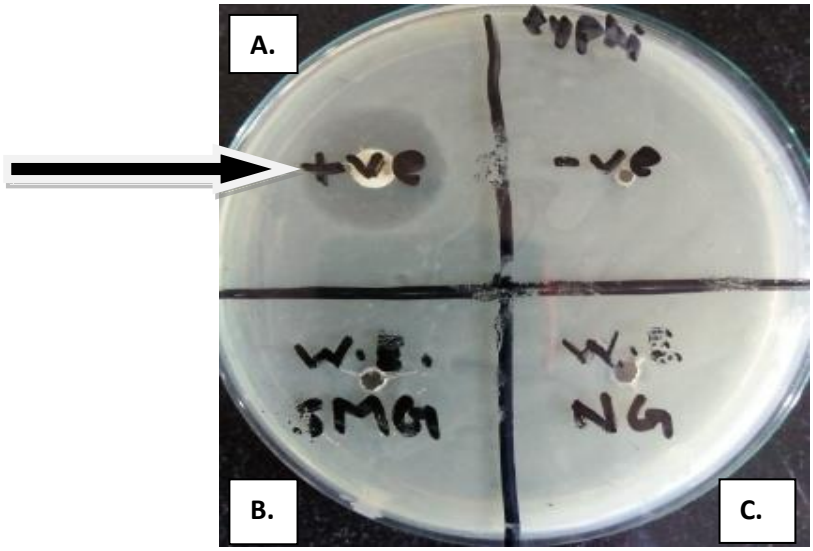

Figure4.2(a) Positive and negative controls for *Salmonella typhi*

A) Antibiotic disc Tetracyclin (Positive control)
B) Water C) Ethanol (Negative control)

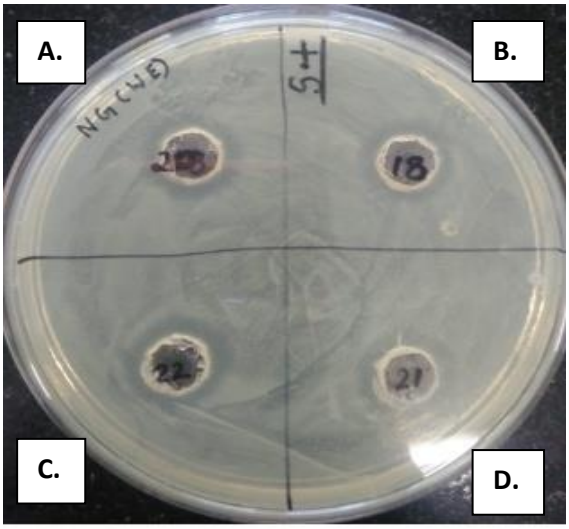

Figure4.2 (b) *Allium sativum* (Water Extract)

A) 20mg/mL B)18mg/mL C)22mg/ML D)21mg/mL

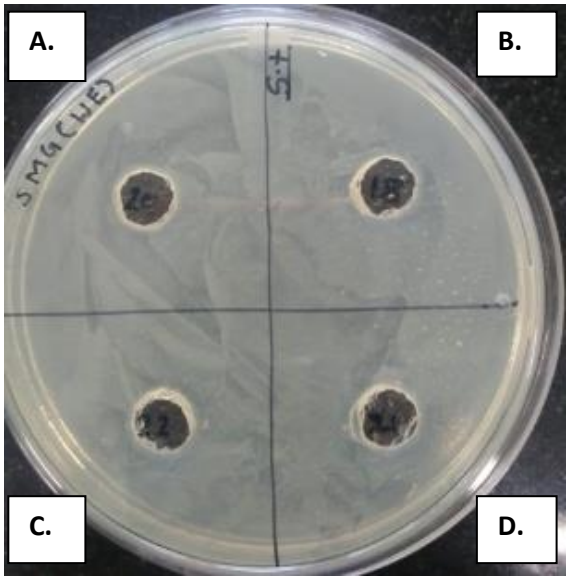

Figure4.2(c) Different concentrations of *Allium schoenoprasum* (Water extract)

A) 20mg/mL B)18mg/mL C)22mg/mL D) 21mg/mL

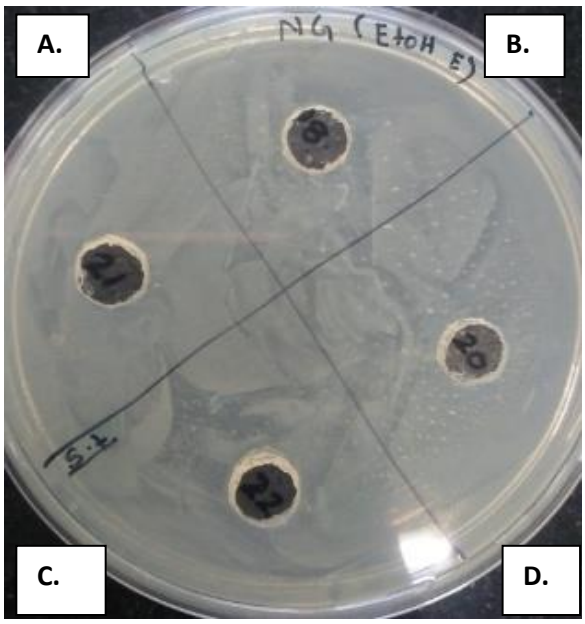

Figure4.3 (d) Different concentrations of *Allium sativum* (Ethanol extract)

A) 21mg/mL B)18mg/mL C)22mg/mL D) 20mg/mL

4.3) Results of experiment with *Staphylococcus aureus*

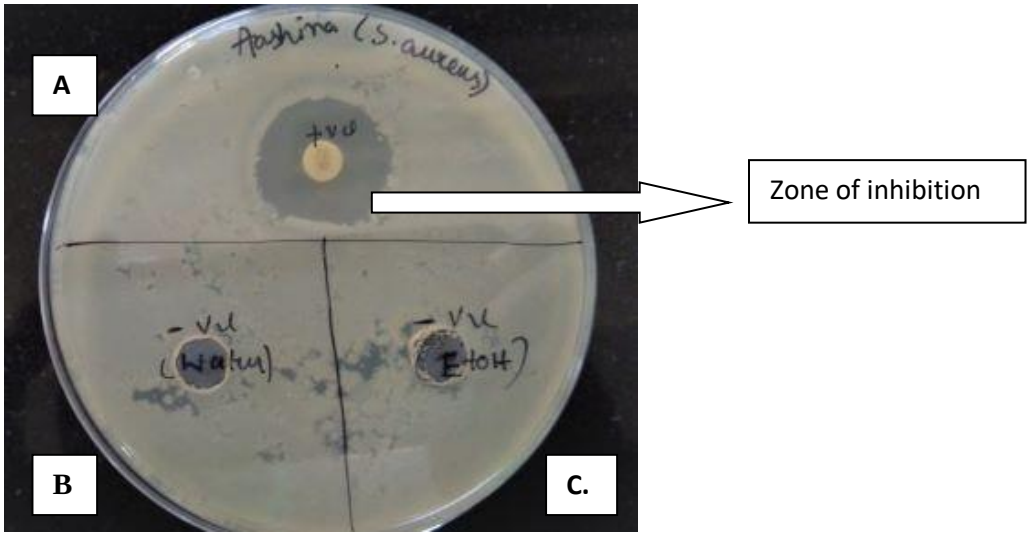

Figure4.3 (a) Positive negative control of Staphylococcus *aureus*

A) Tetracycline (Positive control)
B) Water C) Ethanol (Negative control)

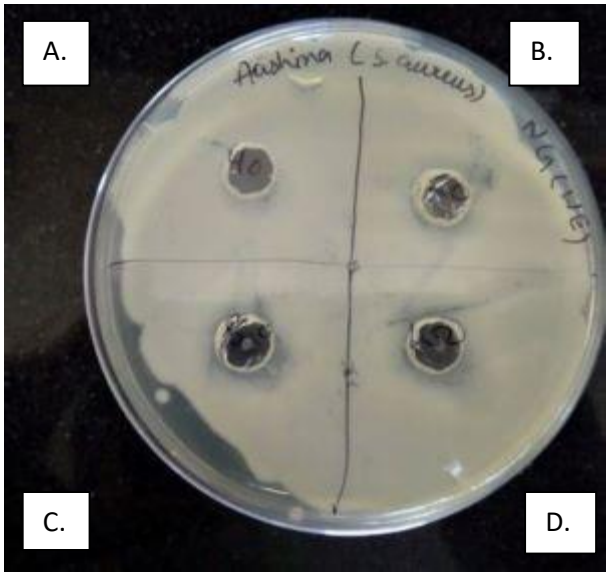

Figure4.3 (b) Different concentrations of Water extract of *Allium sativum*

A) 40 mg/mL B) 60 mg/mL C) 80mg/mL D) 100mg/mL

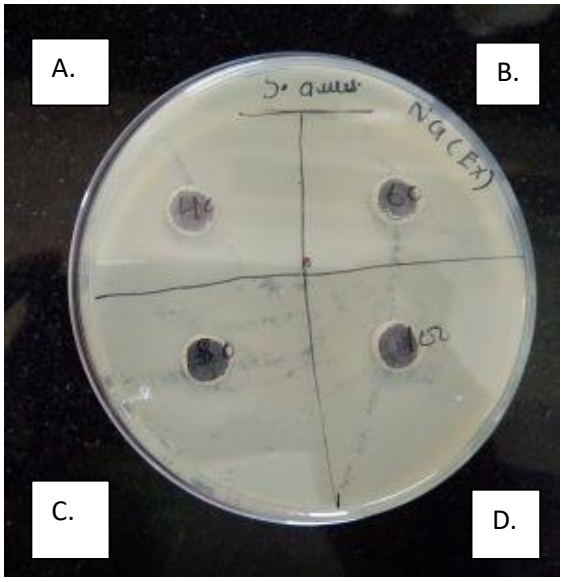

Figure4.3 (c) Different concentrations of Allium *sativum*

A) 20mg/mL B) 40mg/mL C) 80mg/mL D) 100mg/mL

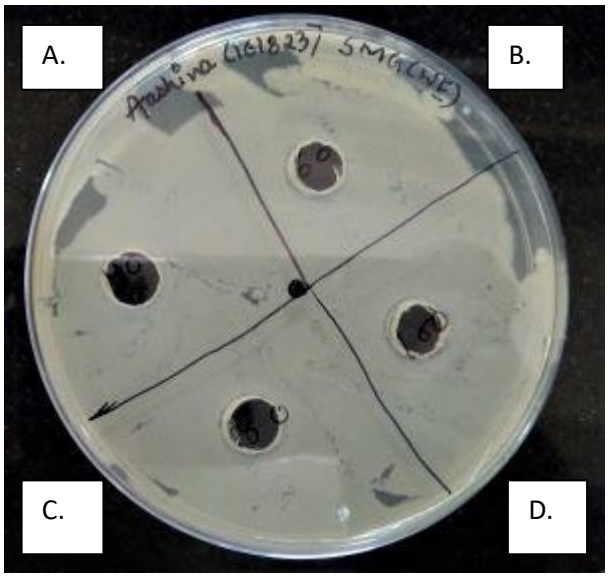

Figure4.3 (d) Different concentrations of extract *of Allium schoenoprasum*

A) 20mg/mL B)40mg/mL C)80mg/mL D) 100mg/mL

4.4) Result of experiment on *Bacillus subtilis*

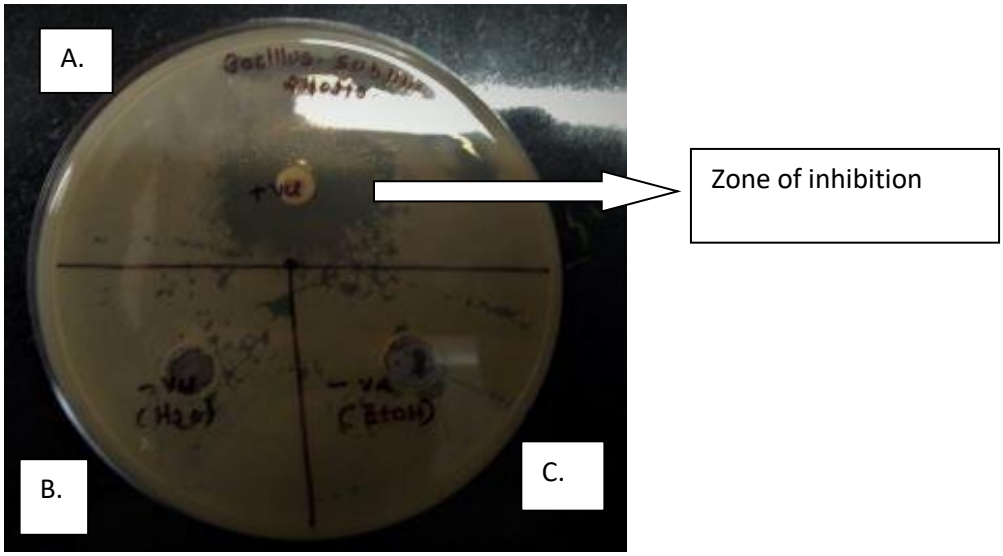

Figure4.4 (a) Positive and Negative controls of *Bacillus subtilis*

A) Gentamicin Positive control
B) Water C) Ethanol Negative control

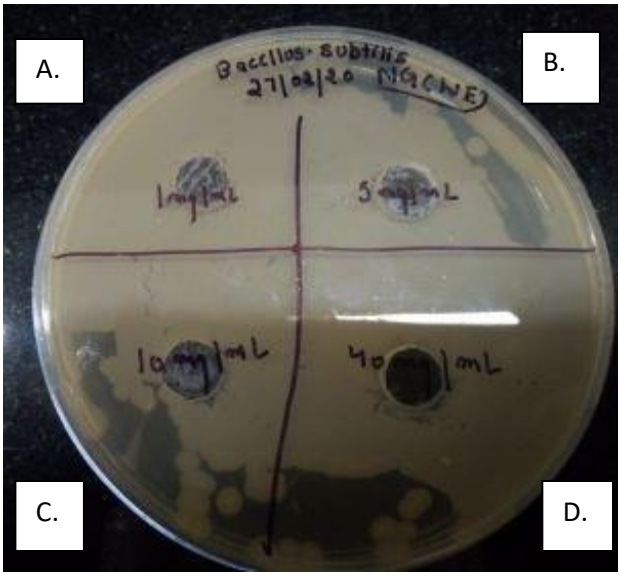

Figure4.4 (b) Different concentrations of water extract of *Allium sativum*

A) 1mg/mL B) 5mg/mL C) 10mg/mL D) 40mg/mL

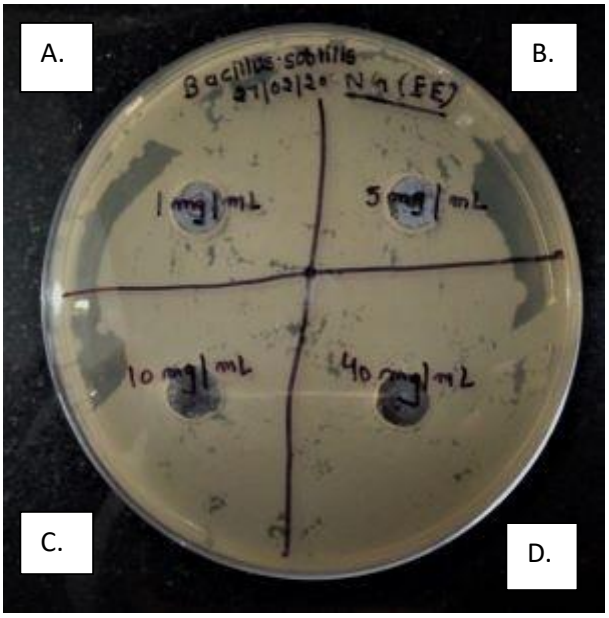

Figure4.4 (c) Different concentrations of ethanol extract of *Allium sativum*

A) 1mg/mL B)5mg/mL C)10mg/mL D)40mg/mL

Figure4.4 (d) Different concentrations of water extract of *Allium schoenoprasum*

A) 1mg/mL B)5mg/L C) 10mg/mL D) 40mg/mL

4.5) Result of experiment on *Mycobacterium smegmatis*

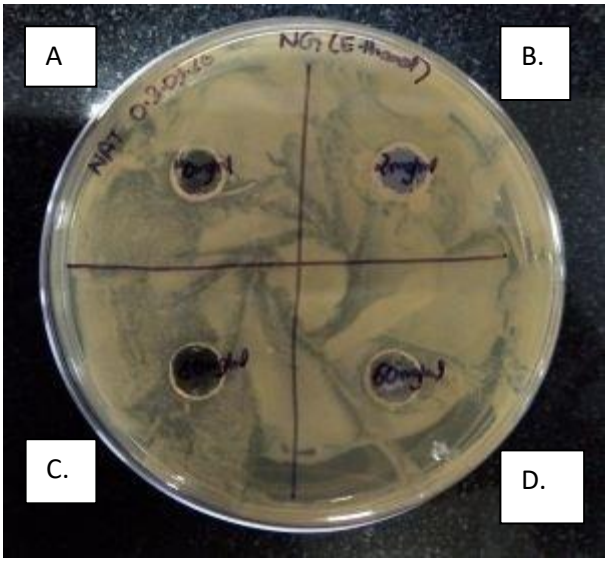

Figure4.5 (a): Different concentrations of ethanol extracts of *A. sativum*

A) 2mg/mL B)40mg/mL C)60mg/mL D) 80mg/mL

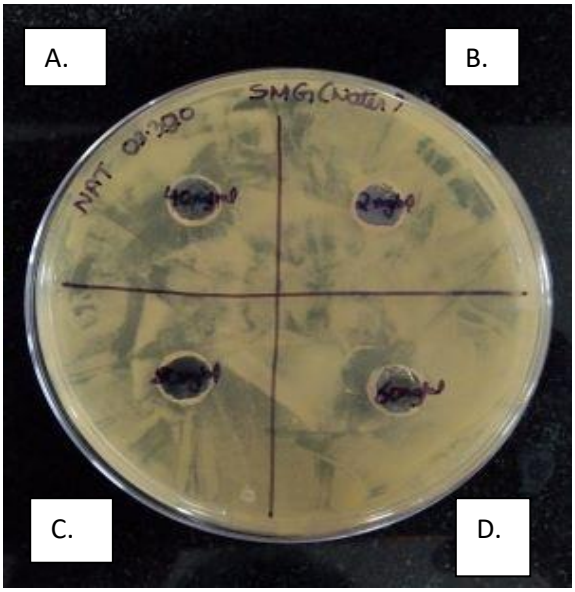

Figure4.5 (b) Different concentration of water extracts of *Allium schoenoprasum*

A) 2mg/mL B)40mg/mL C)60mg/mL D)80mg/mL

The pictures depicting the results of:

Positive and negative controls of *Mycobacterium* and activity of water extract of *Allium sativum for Mycobacterium* would be inserted later as no access of those pictures is available currently.

CHAPTER 5

RESULT AND DISCUSSION

5.1) For *Salmonella typhi*

- **Water extract of *Allium sativum* showed activity against *Salmonella typhi* at all the concentrations** whereas **water extract of *Allium schoenoprasum* did not show any activity against *Salmonella typhi* at any concentration.**
- **Ethanol extract** of *Allium sativum* **did not show any activity** against *Salmonella typhi* at any concentration.
- Ethanolic extract of *Allium schoenoprasum* was not tested because the ethanolic extract of *Allium schoenoprasum* was extracted in very small amounts.

5.2) For *Staphylococcus aureus*

- **Water extract *(Allium sativum*) – No activity**
- **Ethanolic extract (*Allium sativum*)- No activity**
- **Water Extract (*Allium schoenoprasum*) – No activity**
- Ethanolic extract of *Allium schoenoprasum* was not tested because the ethanolic extract of *Allium schoenoprasum* was extracted in very small amounts.

5.3) For *Bacillus subtilis*

- **Water extract (*Allium sativum*) – No activity**
- **Ethanolic extract (*Allium sativum*)- No activity**
- **Water Extract (Allium *schoenoprasum*) – No activity**
- Ethanolic extract of *Allium schoenoprasum* was not tested because the ethanolic extract of *Allium schoenoprasum* was extracted in very small amounts.

5.4) For *Mycobacterium smegmatis*

- **Water extract (*Allium sativum*) – No activity**
- **Ethanolic extract (*Allium sativum*)- No activity**
- **Water Extract (Allium *schoenoprasum*) – No activity**
- Ethanolic extract of *Allium schoenoprasum* was not tested because the ethanolic extract of *Allium schoenoprasum* was extracted in very small amounts.

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