A STUDY ON THE ANTIMICROBIAL ACTIVITY OF GARLIC EXTRACT AGAINST *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS*

DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE AWARD OF THE DEGREE

OF BACHELOR OF SCIENCE

IN BOTANY

BY

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ERNAKULAM

2023-24

CERTIFICATE

This is to certify that the dissertation entitled 'A study on the antimicrobial activity of garlic extract against *Escherichia coli and Staphylococcus aureus*' submitted, in partial fulfillment of the requirements for the award of the degree of Bachelor of Science in Botany is an authentic work carried out by Aarsha P Sanjay (Reg. No. AB21BOT001)

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DECLARATION

I hereby declare that the work is being presented in the dissertation, entitled **A STUDY ON THE ANTIMICROBIAL ACTIVITY OF GARLIC EXTRACT AGAINST *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS*,** in fulfilment of the requirements for the award of the degree of Bachelor of Science in Botany and submitted to St. Teresa's College (Autonomous), Ernakulam is an authentic record of my own during B.Sc period under the supervision of Ms I K Nishitha.

The matter embodied in this dissertation has not been submitted by me for the award of any other degree of this or any this or any other University/Institute.

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Place: Ernakulam
Date: 18|03|2024

Signature of the candidate

ACKNOWLEDGEMENT

I wholeheartedly express my gratitude to Ms I K Nishitha, Head of the department of Botany, St.Teresa's College, for suggesting the topic and guiding and inspiring me through the period of this work.

I am thankful to Dr. Liza Jacob, Associate Professor and Head, Department of Botany, for providing us with all the facility and support for carrying out the project. I also thank all the faculty members of the Department for their support and constant upliftment throughout the course of study.

I express our sincere thanks to Ms. Tiya K. J. Research Assistant and Consultant, Teresian Instrumentation Centre, for helping us carryout the antimicrobial studies in the instrumentation facility of the College. I also thank all the non-teaching staff of the Department of Botany and other supporting staff of the Science block for the help rendered during our work.

I thank our fellow project coworkers for the invigorating discussion and coordination we had throughout the course of study.

I express our gratitude to my classmates who played an excellent role in showering their love, friendship and generosity.

I convey our ardent gratitude to our parents for their reckless support and guidance directly and indirectly. Finally I thank god almighty for guiding us through the right path.

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CHAPTER 1

INTRODUCTION

The antimicrobial activity of garlic is mainly due to the presence of most biologically active compound that is Allicin. Allicin (diallyl-thiosulfinate) has sulfhydryl modifying activity and it is capable of inhibiting sulfhydryl enzymes. Allicin is not present in raw garlic and they are formed rapidly by the action of the enzyme, allinase.

Garlic has been used for thousands of years due to its medicinal properties. It is having wide spectrum of action. Alone with antibacterial, antiviral, antifungal, antiprtozoal, it is also shows effects on the cardiovascular and immune systems. Garlic shows antimicrobial activity agaist a wide range of Gram-positive and Gram-negative bacteria.

Acacia (19 species), Cinnamomum (15 species), Salvia (11species), Thymus (11 species), Lantana, Tarmarind, Phyllanthus, Jatropha are some of the plants showing antibacterial property.

1.1 HISTORY OF GARLIC

Garlic (*Allium sativum*) member of Alliaceae family, is a widely used as spice. It originated in Central Asia, in the region that covers modern day Kazakhstan, Kyrgyzstan, Tajikistan and Uzbekistan. From there it spread to other parts of the world through trade and exploration. It is believed that garlic was first cultivated by the Sumerians, 5000 years ago in the shore of Mediterranean sea.

Garlic has been highly valued highly valued for its medicinal properties cultivated and used worldwide. The presence of many biologically active substances in garlic, especially in its underground bulb, makes the plant suitable for use in alternative medicine.

Garlic can be provided in the form of capsules and powders, as dietary supplements and thus differ from conventitional food or food ingredients(*Magrys et al.,2021*). Garlic is also effective against antibiotic resistant organisms. Garlic extract has antimicrobial activity against oral

bacterial species, particularly gram-negative species (*Kshirsagar et al.,2018*) Allicin is the main compound in garlic which is responsible for its antimicrobial activity.

1.2 HABITAT

Garlic plant can grow in various habitat. It is cultivated in drained soil and requires full sun exposure. Garlic can tolerate a wide range of soil types but extremely acidic soil as well as heavy soil is not suitable for this crop. It mainly prefers loamy soil with good organic matter content.

It is a crop that requires very low maintenance but requires regular watering. It is important to provide adequate moisture and proper drainage facilities for the optimal growth of the crop. This also helps to increase the resistance of the crop towards various diseases and pest. About 12-18 degree Celsius is the temperature requirement of garlic during its vegetative growth.

1.3 MEDICINAL USES OF GARLIC

Heath benefits that are associated with garlic are attributed to its anticancer, anti-inflammatory, antifungal, antiviral, and antibacterial activity (*Bhatwalkar et al., 2021*). Garlic extracts have also been used in research on diabetes, which confirmed its effectiveness in reduction of blood glucose levels (*Ohaeri 2001; Eidi et al. 2006*). For the last few decades, the incidence of microbial infections caused by multidrug-resistant pathogens has increased dramatically.

Multidrug resistance (MDR), defined as the ability of a microorganism to resist the action of an administered antimicrobial drug despite earlier sensitivity to the substance, has become a significant public health threat, as almost all antimicrobial agents available are subject to the problem (*Jyoti et al. 2014; Magiorakos et al. 2011; Gaekwad and Trivedi 2013*). Garlic is know to show antimicrobial activity against both gram-positive and gram-negative bacteria.

Extract from the underground garlic bulb, especially its compound allicin (diallyl-thiosulfinate), inhibits the growth of many species of Gram-positive and Gram-negative bacteria (*Yoshida et al. 1999; Tsao and Yin 2001; Bakri and Douglas 2005*). Therefore, garlic is an excellent alternative to the antibiotics. Garlic is best know to boost immune system by stimulating certain cells like macrophages, lymphocytes, natural killer (NK) cells, dentritic cells and eosinophils. It lowers calory, cholesterol, blood pressure. Garlic compounds exhibit

multiple types of antibacterial activity and have enormous potential to be developed into novel antibacterial agents (*Bhatwalkar et al., 2021).*

BOTANICAL CLASSIFICATION OF GARLIC IS AS FOLLOWS:

Kingdom	Plantae
Order	Asparagales
Family	Amaryllidaceae
Subfamily	Allioideae
Genus	Allium
Species	A.sativum

1.4 OBJECTIVES

1. Extraction of garlic in polar and non polar solvents (ethanol and hexane).

2. Identification of antimicrobial activity against Gram-positive and Gram-negative bacteria (*Escherichia coli , Staphylococcus aureus).*

CHAPTER 2

REVIEW OF LITERATURE

2.1 REVIEW ON PREVIOUS WORKS

Previous studies on *Allium sativum* conclude that it is known to act as antibiotic and no resistance to it has been reported. It is know to be resistant against a wide spectrum of bacteria, fungi and viruses. Various tests has revealed that the antimicrobial activity of garlic is totally depended on Allicin compound . This compound present in garlic is three times more effective on gram-positive bacteria than gram-negative bacteria.

It has beneficial effects on cardiovascular and immune system. The dosage of garlic as an antibiotic varies from person to person. The best way of consuming garlic is by eating them on an empty stomach. This is because bacteria is exposed and cannot defend itself from resisting to its power. Some people shows allergic reactions to garlic, taking garlic supplements may increase the risk of bleeding in some people. Where as it can prevent cold and flu virus in others.

Eating raw garlic can protect an individual from cough, fever and cold illness. It is also said that green sprouts from the top of bulb is caused due to improper storage such garlics are not good for eating. Sprouted garlic can be eaten but make sure that you remove the sprouts as they are bitter in taste. Due to the anti-platelet properties it is known to be a blood thinner (prevents the platelets from clumping together to form a clot). Anti-platelets are taken by people who had heart attack and stroke.

Current studies on garlic is that it showed strong anti-cancer activity, particularly digestive tract tumors, garlic supplements to reduce blood pressure in people with high blood pressure. Researches linked this effect to a16-40 percent risk of experiencing cardiovascular events.

2.2 *Allium sativum* (commonly known as Garlic)

Studying the antimicrobial properties of garlic helps us understand how garlic can inhibit the growth of bacteria. Garlic can fight against various bacteria, viruses, fungi, and parasites. Hence garlic is used as a natural remedy for infections and illnesses. Garlic has been traditionally used as a home remedy to treat various infections, because of its antimicrobial and antiviral properties, one such example is use of garlic against warts. Garlic is crushed and directly applied on warts to eliminate them over time.

Garlic is also used to treat various illnesses. Some of them are listed below :

1. Garlic against Cold and Flu : Consuming raw garlic may alleviate cold and flu symptoms due to its antimicrobial properties.

2. Garlic for Ear Infections : Garlic oil is used to treat ear infections. It is prepared by crushing few cloves of garlic and mixing them with olive oil.

3. Garlic against Acne : Antimicrobial properties of garlic help to reduce acne. Crushed garlic is directly applied on to the acne.

4. Garlic for preparing face mask : Crushed garlic is mixed with honey and this is applied to the face for glowing skin.

5. Garlic for high Blood Pressure : Including garlic supplements or incorporating garlic in our diet can reduce or lower blood pressure.

6. Garlic for reducing Acidity : It may help to reduce acidity due to its ability to regulate digestive processes by promoting the production of digestive enzymes and gut bacteria.

Garlic is also believed to lower cholesterol and improving circulation. Hence it reduces the risk of stroke and heart diseases. Its beneficial effects on cardiovascular health attributed to its ability to improve blood circulation. Garlic contain a chemical compound known as allicin which is an antioxidant, it can reduce the risk of chronic diseases like cancer. Garlic is associated with various health benefits, such as boosting immune system, having potential anti-inflammatory and antioxidant effects. Modern studies on garlic explores health benefits.

Extracts of garlic has been found to help in wound healing by promoting tissue regeneration, reducing inflammation and preventing infection.

Hence modern studies on garlic continues to support the traditional use of garlic in medicine, its potential as a remedy for various health condition.

2.3 *STAPHYLOCOCCUS AUREUS*

It is a gram positive bacteria that cause a variety of diseases. Infections caused by this pathogen are common both in community acquired and hospital acquired settings. The treatment is challenging due to the introduction of multi-drug resistant strains such as MRSA (Methicillin-resistant *Straphylococcus aureus*), VRE (Vancomycin- Resistant Enterococci). S. aureus does not cause infection on healthy skin normally. But if they are allowed to enter the internal tissues or blood, these organisms can cause a wide variety of potentially serious infections and diseases. (*Taylor, 2023).* It is a common commensal of humans and its habitat is moist squamous epithelium of the anterior nares. Healty individuals are having small risk. But finite risk of contraction an invasive infection caused by S. aureus, and this risk is increased among the carriers. (*Timothy J. Foster,2004).* Its activity can range from harmless colonization on the skin and soft tissue infections, pneumonia and bloodstream infections.

Treatment normally involves antibiotics, but some strains have developed resistance, making management challenging. There have been many studies on S. aureus due to its importance on medical field. These studies cover epidemiology, antibiotic resistance, virulence factor, pathogenesis and various treatments. Some of the recent studies on this organism focus on genomic analysis to understand the evolution and spread of antibiotic resistant strains. Non pathogenic strains of S. aureus may be used for research purposes such as the study of bacterial genetics, physiology. Some of the strains of S. aureus have been engineered to produce useful compounds such as enzymes, proteins. They are also used for biotechnological applications.

However biotechnological , medical and industrial application of these bacteria are limited as compared with other. Some of the species of Straphylococcus such as *Straphylococcus carnosus* is used in food industry for fermenting and flavoring purposes. This bacterium helps in breaking down proteins and contributes to characteristic taste and texture of fermented meats. S. aureus plays a crucial role in several ways like:

1.Antibiotic development : Development of vaccines targeting specific strains to prevent infections or reduce its effects.

2.Drug Testing : These organisms are used in laboratories for testing efficacy and safety of pharmaceutical compounds (antibiotics and antiseptics).

3. Biofilm Research : Understanding the concept of biofilm formation helps in developing strategies to disrupt biofilms, which is crucial for treating serious infections related with S. aureus.

4.Virulence factor targeting : It involves developing drugs aimed at weakening bacterium ability to cause disease.

2.4 *ESCHERICHIA COLI*

It is a gram-negative bacillus that is a causative organism of many diarrheal diseases like travelers diarrhea and dysentery. E. coli is the most common pathogen leading to uncomplicated cystitis and internal illnesses that includes pneumonia, bacteremia and abdominal infections such as bacterial peritonitis. Illness caused by these organisms have a burden on patients. So appropriate recognition and treatment is required *(Mueller et al, 2023)*.

E coli. is the most common bacterium found in human gastrointestinal tract and is not virulent in this setting. Virulent E coli. affects wide range of eukaryotic cellular processes like cell signaling, ion secretion, protein synthesis, mitosis, cytoskeletal and mitochondrial functioning *(kaper et al, 2004)*.

The most important application of E coli. in the medical field involves the preparation of artificial human insulin by Biotechnology. Importance of E coli. lies in various domains like:

1.Biological Research : E coli. is most commonly used in research studies because of ease of manipulation, and rapid growth rate. It is used for understanding biological processes such as gene regulation, DNA replication, and protein expression.

2.Biotechnology : E coli. is widely used for the production of recombinant proteins, enzymes and pharmaceuticals. For the large scale production of compounds like insulin, growth hormones and vaccines.

3.Environmental aspects : E coli. is used particularly in checking water quality.

4.Medical Research and Diagnostics : Some strains of E coli. are pathogenic it may cause a wide range of infections in human body including urinary tract infections, gastrointestinal illnesses, and bloodstream infections. Studies done on these strains has led to advances in diagnostic methods, development of antibiotics and strategies for control of various infections.

5. Food industry : E coli. can be harmful in food if it contaminates the food. But some of its strains are used in food production, like cheese making and yogurt fermentation. They can also provide flavor and texture to the food products.

Contamination can occur in food industry through improper handling of food products, contaminated water or contact with fecal matter during its production.

CHAPTER 3

MATERIALS AND METHOD

The influence of garlic bacterial growth was studied with the help of both gram positive bacteria and gram negative bacteria i.e Staphylococcus aureus and E coli.

The following materials are used for the study of antimicrobial effect of garlic:

1. Garlic 40g
2. Ethanol
3. Hexane
4. Mortar and pestle
5. Soxhlet apparatus
6. Beaker
7. Dimethyl sulfide (DMSO Solution)
8. Test tubes
9. Nutrient broth
10. Agar agar
11. Autoclaving machine
12. Incubator
13. Petriplate
14. Cotton swab
15. Tip
16. Pipette
17. Negative control
18. Positive control
19. Gel puncher

Antibiotics is taken as positive control and DMSO is taken as negative control.

3.1 Fresh garlic was peeled and weighed for 20g. It was then crushed by using mortar and pestle. Crushed garlic is wrapped with filter paper so that no pieces of garlic stuck inside the Soxhlet apparatus.

Soxhlet apparatus is used for the extraction of garlic. A Soxhlet apparatus consist of mainly 3 parts : Percolator (boiler, where solvent circulates), a thimble (made of thick filter paper), and a siphon (it periodically empties the thimble).

The garlic which is covered with the filter paper is now placed inside the thimble and about 500ml of ethanol is poured inside the round bottom flask. The temperature of the apparatus is fixed at 70 degree Celsius. Ethanol is a highly volatile liquid, extraction process undergoes 7-8 cycles. As the ethanol evaporates it takes the essence of garlic along with it. When the vapor reaches the condenser which is provided with water inlet and outlet, it condenses and reaches the thimble. And when it reaches a particular level the liquid moves in to the round water flask through the siphon. This process is repeated 8 times and a brown color extract of garlic was collected .

This brown solution was evaporated with the double boiling method and allowed to cool. Then the residues of garlic was then mixed with 10ml DMS solution.

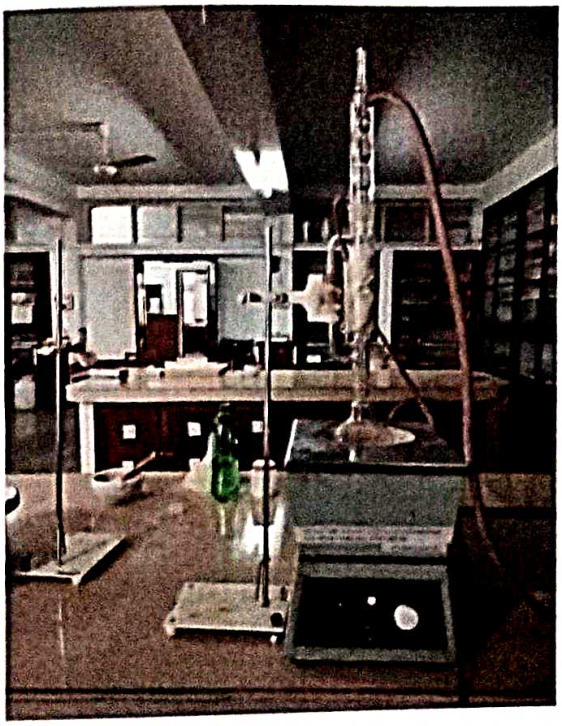
Weight of this solution along with the container is 9.2g (weight of the contain er 0.9g).

Again 20 grams of garlic is taken and extracted with hexane by the soxhlet apparatus. Temperature is set at 70 degrees Celsius. As hexane is more volatile than ethanol, it takes lesser time to evaporate and condense as compared with ethanol. About 15 cycles was completed, no particular color change occurs in the solution. The collected extract was cooled and evaporated by using double boiling method. It was then mixed with 10ml DMS solution.

Weight of the solution along with the container is 7.8g (weight of the container 0.9g).

After the garlic extract is mixed with DMSO, it is sorted as 100%, 50% and 25% of concentrations of garlic extract from hexane and ethanol. Each test tube was marked with the concentrations.

The antibacterial activity is completed in three consecutive days. Three different procedures are done in three different days and it involve inoculation of bacteria on the first day, growth of bacteria on the second day and activity of desired bacteria (E coli. and Staphylococcus aureus) towards the extract on the third day.



3.2 PREPARATION OF NUTRIENT MEDIA (Day 1)

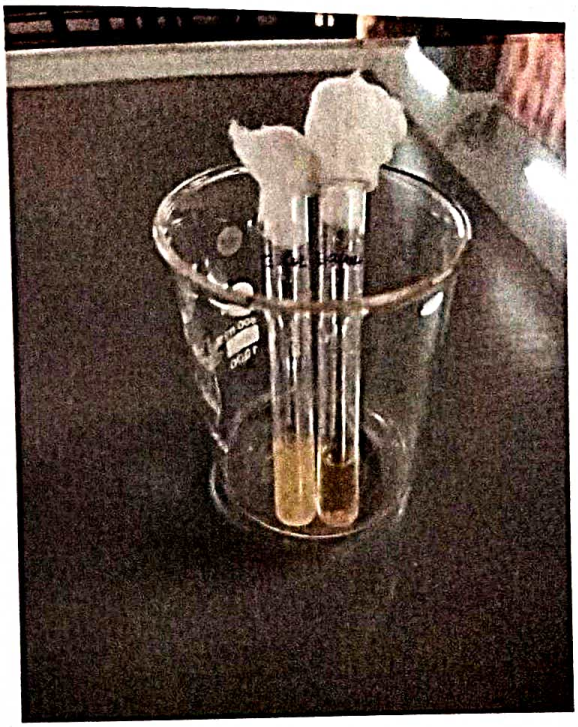
(LIQUID MEDIUM PREPARATION)

The nutrient broth was prepared by dissolving 1.3gm of nutrient broth in 100ml distilled water. Test tubes were filled with 5ml of nutrient broth and were sterilized using an autoclave ((

15LBS pressure, 121 degrees Celsius for 15-20 minutes). After sterilization it is kept in an incubator at 37 degrees Celsius. Over night incubation is done.

Day 2 (SOLID MEDIUM PREPARATION)

Nutrient agar media was prepared by mixing 1.3gm of nutrient broth and 2gm of agar agar in 100ml distilled water. (by using 100ml distilled water we can prepare 5 plates, almost 20ml for one plate.) The media was autoclaved and 20ml each poured into sterile petri plates under aseptic conditions. Wait for 15 minutes.



3.3 SWABBING (WELL DIFFUSION METHOD) 2nd Day

A lawn culture of each bacterium was prepared using sterilized cotton swabs. A sterilized swab was dipped into the bacterial suspension and moved side to side from top to bottom leaving no space uncovered. The plate is rotated to 90 degrees and the same procedure was repeated so that the entire plate was coated with bacteria. Four plates was taken and two of them was coated with E coli. and other two plates with S aureus.

Once the law is prepared, wells of 6nm diameter was made by using a gel puncher. Before making wells with the gel puncher it is also sterilized. The wells are labelled with a permanent marker for 100%, 50%, 25% , positive and negative control. Sample of each concentration is filled into the well according to the label with the help of an electric pipette.

The samples was loaded in such a way that it is not overflowing from the well. After loading the samples positive control that is a standard antibiotics is kept and also a negative control that is DMSO was loaded into one of the well. And it was kept undisturbed overnight.

CHAPTER 4

OBSERVATION AND RESULT

Antimicrobial property of garlic is the study conducted to observe the effect of garlic on different bacterial stains. Here we used both gram-positive as well as gram-negative bacteria that is *Staphylococcus aureus* and *Escheriachia coli* . Garlic extract was taken using the soxhlet apparatus and converted to 100%, 50% and 25%. To observe the effect of garlic in these concentrations. Then bacteria prepared on in the agar plate for the test. The extract of different concentration that is prepared is loaded in the well created on the agar plate by using a micropipette. It was kept over night to observe the result. We got the result in E coli. Bacteria. Zone of inhibition on antibiotics- Gentamicin we got was almost 1.9cm, which shows the plate is working. The negative control was DMSO to show that the zone is produced only due to the effect of allicin present in garlic.

Table 1: Effect of garlic extracts on *E. coli* and *S. aureus* in polar and non-polar extracts

Concentration	**E. coli** Zone of inhibition (in cm)		**S. aureus** Zone of inhibition (in cm)	
	Ethanol	Hexane	Ethanol	Hexane
25%	0.8	0.9	-	-
50%	1	1	-	-
100%	1.4	1	-	-

Zone of inhibition was formed in all the concentrations except the negative control. From this we can conclude garlic shows antimicrobial activity against E coli. bacteria. In the case of S. aureus bacteria the zone of inhibition was formed only in the positive control that is in place of antibiotics. This shows garlic does not show its activity against S. aureus bacteria.

RESULT WITH *E. COLI*

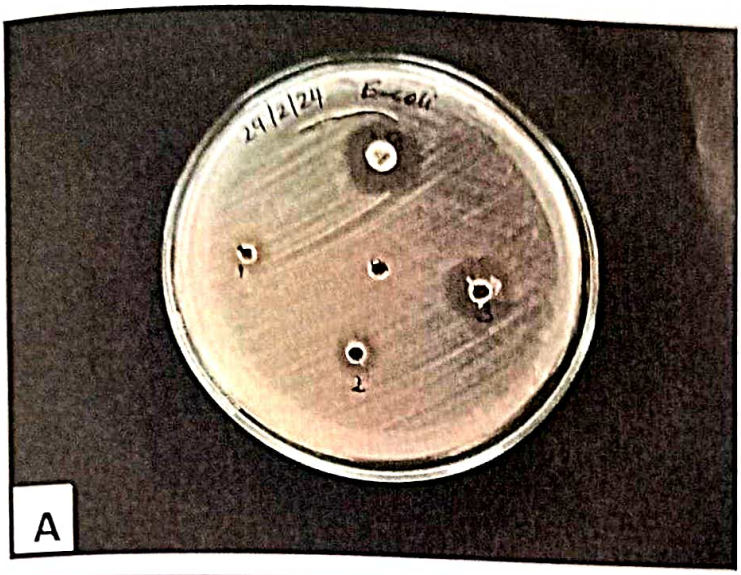


Fig A shows the plate with gram-negative bacteria(*E.*coli) with concentrations 100%,50% and 25% extracted with polar solvent ethanol.

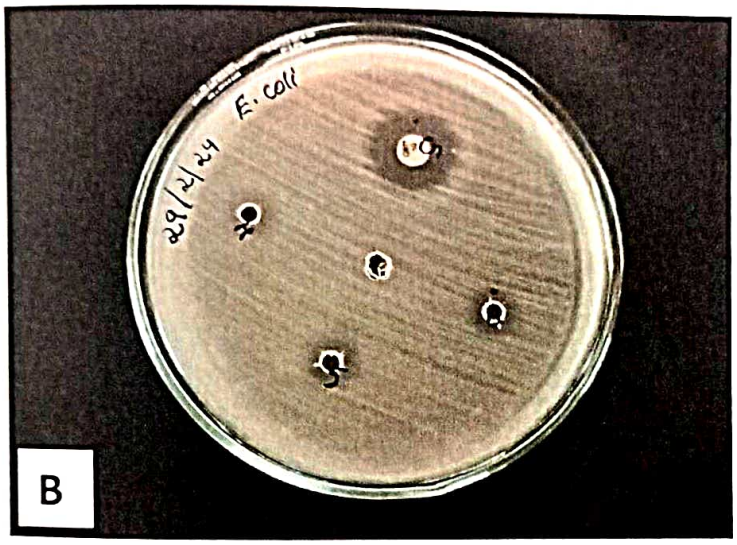


Fig B shows the plate with gram-negative bacteria (*E.coli*) with concentrations 100%,50% and 25% extracted with non-polar solvent hexane.

RESULT WITH *S. AUREUS*

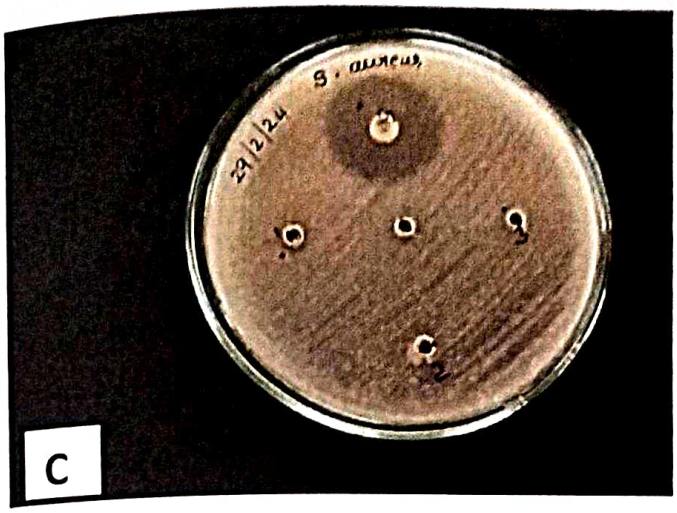


Fig C shows the plate with gram-positive bacteria (*S.aureus*) with concentrations 100%,50% and 25% extracted with polar solvent ethanol.

Fig D shows the plate with gram-positive bacteria (*S.aureus*) with concentrations 100%,50% and 25% extracted with non-polar solvent hexane.

CHAPTER 5

DISCUSSION

Antimicrobial activity of garlic extract against *E.coli and S.aureus* shows that garlic has the ability to inhibit the growth of *E.coli* bacteria in the given concentrations that is 100%, 50% and 25%. According to our results it is observed that the zone of inhibition is more for 100% concentration of garlic extract that is1.4cm. Garlic extract was taken through soxhlet apparatus by using ethanol and hexane as the solvents. In both the cases garlic extract shows higher activity in 100% concentration. Where as we observed that it does not show any activity against *S.aureus* bacteria as no zone of inhibition is produced.

The phytoconstituents of garlic have long been known and its antibacterial properties have been widely reported (Roy et al., 2006). The antimicrobial activity of extracts of garlic have long been linked to the presence of some bioactive compounds. These secondary metabolites also serve to protect the plants themselves against bacterial, fungal and viral infections (El-Mahmood and Amey, 2007). These bioactive compounds are known to work synergistically to produce various effects on the human and animal subjects (Amagase, 2006).

The sensitivity of the *E. coli* to garlic was more or less the same in 25% and 50% concentrations of ethanolic and n-hexane extracts, but at 100% concentration the ethanolic extract had a larger inhibitory zone for *E. coli*. According to Cheesbrough (1984) an antimicrobial agent which the diameter of the zone of inhibition is above 3mm, the organism is said to be sensitive but if it is 2mm or less than that, the organism is said to have resistant against the particular agent.

The phytochemical and antimicrobial property of garlic extracts against three bacterial isolates (*Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*) was studied by Garba Iet al., 2014. The aqueous and methanol extracts showed potency against *E. coli* with the inhibition zones of 24.0mm at 200mg\ml and 23.0mm at 200mg\ml respectively. In the present study also E. coli was inhibited by ethanolic and hexane extracts.

According to the results of Khashan, who assessed the antibacterial activity of extract of garlic against *S.aureus* bacteria found that concentrations of garlic extract ranging from 10 to

20mg\mL were unable to inhibit the growth of bacteria. *Escherichia coli* was least suspectible and shows minimum sensitivity when tested against 40mg\ml with an inhibition zone of 14.2-0.8mm and 16.3-0.7mm using agar disk diffusion and agar well diffusion methods.

In a study on *Staphylococcus aureus, Escherichia coli and Klebsiella pneumoniae,* Abidullah et al. (2021), examined the susceptibility of the test organisms against the garlic extract and found that isolates of these bacteria were sensitive to the concentration of 10micro litre garlic of agar media in using diffusion method. In addition 2nd largest clear zone on *E.coli* that is 27mm concentration against among microorganism.

The extract of garlic contains a varied range of antimicrobial/antibacterial potential which are effective against Gram-negative organisms (*E. coli* and *K. pneumoniae*) and Gram-positive bacterial *Staphylococcus*. According to Abebe, 2003, the components which bring about this effect are the allicin which mainly inhibits the growth of bacteria by inhibiting the DNA and protein synthesis partially along with RNA inhibition synthesis as the primary target.

The present study result also suggest that garlic exhibits a large clear zone against *E. coli*, comparable to the antibiotics used in the study. Hence its use as a common remedy is justified.

CHAPTER 6

CONCLUSION

Garlic is a herb which is widely used all around the world in food and as a flavouring agent. The bulb of te plant is the most commonly used part.Garlic is a strong antimicrobial agent, it inbihits the growth of both gram-positive and gram-negative bacteria.The antibacterial property of garlic is purely due to the presence of an active compound present in it that is allicin.Raw garlic does not contain the presence of allicin, it is formed by the action of enzyme allinase.

The study was done to observe the antimicrobial activity of garlic on both gram-positive and gram-negative bacteria. *Escherichia coli* was taken as the gram-negative bacteria and *Staphylococcus aureus* was taken as the gram-positive bacteria. The garlic extract was taken from raw garlic by using soxhlet apparatus in both polar(Ethanol) and non polar(Hexane) solvents.Extract was the evaporated by using double boiling method to remove the solvents.Then it was dissolved in DMSO solution and converted into different concentrations(100%,50% and 25%).The antibacterial test was done by using well diffusion method and the result was obtained on the 3rd day.

In the present project zone of inhibition was observed only with gram-negative bacteria (*E.coli*). And no zone was observed with the gram-positive bacteria(*S.aureus)* . The antibiotics gentamicin showed a zone of inhibition of 1.9cm which shows that the plate is working. The negative control was DMSO solution which didn't produce any zone. It shows us that zone of inhibition was produced only due to the effect of allicin present in garlic. More activity was shown by the 100% concentration of garlic extract.

The present study claims that Garlic is a strong antibiotic agent. And this property of garlic is due to the presence of a compound allicin. Further studies can be done to find the uses of garlic for the prevention of drug resistance microbial diseases.

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