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ANTIMICROBIAL ACTIVITY OF COMMON INDIAN SPICES

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ABSTRACT

The two biggest threats faced by today's medical community are antibiotic resistance and multi drug resistant pathogens. In the present research, to counter these risks, the antimicrobial function of spices has been explored as an alternative to antibiotics. Methanol and acetone extract of 5 Indian spices were screened for antibacterial properties in search of a bioactive compound. The choice of spices as an alternative is based on two fundamental reasons: firstly, since ancient times, plants have been the model source of medicine and, secondly, the growing acceptance by the general population of herbal medicines. To establish the antibacterial properties of the spices, methanolic, ethanolic and acetone extracts were used in this work. The antibacterial activity of five traditional Indian spices against *Vibrio species*, namely cloves, ajwain, fennel, cumin and asafoetida. The results showed that spice methanol extracts (MIC values of 20-100 µl / ml) have a high degree of antimicrobial activity on all test species (inhibition range, 6-16 mm) compared to spice acetone extracts of the same concentration. The findings concluded that these spices contain strong secondary metabolites and they have high antimicrobial activity due to these metabolites and can be used as a good bio-preservative and can also be used for medicinal purposes.

KEYWORDS: Spices, secondary metabolites, extracts, MIC, Zone of Inhibition

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INTRODUCTION

Many beneficial health impacts of traditional food spices have been understood over the past two to three decades. Owing to the growing incidence of new food-borne disease outbreaks caused by pathogenic micro-organisms, there are also new food safety issues. This poses significant challenges , particularly since the use of chemical preservatives and artificial antimicrobials to inactivate or prevent spoilage and pathogenic micro-organism growth is becoming increasingly uncomfortable [1]. In different ways, spices may be applied to foods: as whole spices, as ground spices, or as isolates of their extracts. Some of the most widely used natural antimicrobial agents in foods are spices. In addition to providing flavour and pungent stimuli, the addition of spices in foods provides antimicrobial property [2]. Spices are food additives that are aromatic and pungent, like herbs, and spices can have a major antioxidant effect [3]. Complete equivalent antioxidant and phenolic (Folin-Ciocalteu) capacities of 32 spices were measured [4]. Spices may have antibacterial effects as well, too. Out of 46 measured spice extracts, several showed antibacterial activity against food-borne pathogens. Gram-positive bacteria were generally more sensitive than Gram-negative bacteria resistant. The antibacterial activity of the extracts was closely associated with their phenolic content [5].

MATERIALS AND METHODOLOGY

Materials

In the present study was conducted to find out the antimicrobial activity of five spices

including Clove (*Eugenia caryophyllus*, family Mytraceae), Cumin (*Cuminum cyminum*, family Apiaceae), Asafoetida (*Ferula foetida* L. family Umbelliferae) Fennel (*Foeniculum vulgare* family Apiaceae), Ajwain (*Trachy spermumammi*, family Apiaceae) against pathogenic fungal spices have been recognized for their value of preserving foods and medicinal values due to the presence of bioactive antimicrobial compounds. All spices are purchased from local market of Lucknow region.

Methodology

Sample Collection for Microorganism & Spices

Soil sample was collected from nearby land area. Spices were collected from local market of Lucknow.

Inoculation on Agar Plate Using Spread Plate Technique

- ✓ Firstly, nutrient agar media was prepared and was autoclaved at 15lb/inch^2 at 121ºC.
- ✓ This media was poured into Petri plate and cooled.
- ✓ Then add 200 µl of bacterial sample onto nutrient agar plate and spread it with the help of sterilized L- shaped glass rod (spreader). This work was done in LAF.
- ✓ After spread the plate was incubated at 37 ºC for 24 hours.

Isolation of Single Bacteria Colonies

The theory of this technique is to streak a bacterial suspension until the plate is divided by a single cell. In isolation, each individual cell then grows to create a clone of similar cells known as a "colony." Genetically, the bulk of these cells are similar. However, mutation of a single colony may lead to a low level of mutant cells during development [6].

Protocol (Streak Method)

- ✓ Flame a nichrome loop (3mm across and has 6 cm stem). Allow the loop to cool or cool it by immersion in a sterile area of the medium.
- ✓ The flamed neck is used to remove a loop of cells from an overnight incubated agar media.
- ✓ Streaking the cell at one side of a well dried agar plate (in a manner to make the first arm of a pentagon).
- ✓ Flame the loop and cool it as before.
- ✓ Streak again, starting from one end to make second arm of pentagon.
- ✓ Label each plate to indicate the strain number, genotype of strain, date of culture and name.
- ✓ Wrap the plates with paraffin.
- ✓ Incubate the plate at 37 ºC with the medium facing downwards to reduce the chance of droplets of condensation falling on the medium surface.

Identification of Unknown Bacterial Species

Gram Staining

Gram's staining technique was developed by Dr. Hans Christian Gram. This technique is used for morphological study of unknown bacteria species.

Principle

On the basis of cell wall structure, Gram's staining technique is used to distinguish bacteria. The bacterial culture that turns purple is referred to as gram-positive bacteria, whereas gram-negative bacteria are classified as bacteria that appear pink in colour. Gram-positive bacteria have a thick (90 percent) peptidoglycan cell wall that can maintain the violet-iodine crystal complex that occurs during staining, whereas gram-negative bacteria have a thin (10 percent) peptidoglycan layer [7]. Therefore, gram-positive cells resist decolorization, with many techoic acid cross-links, and gram-negative cells decolorize. This also helps the counter stain safranin to be tolerated by gram-negative cells. Gram positive cells tend to be blue to purple, while gramme negative cells tend to be red to pink in hue [8].

Procedure

- ✓ Prepare and heat-fix the smear.
- ✓ Stain the slide by flooding it with crystal violet with one minute.
- ✓ Pour-off excess dye and wash gently in tap water and drain the slide against a paper towel.
- ✓ Add Gram's iodine for 1 minute by washing with the iodine solution and leaving it on the smear until 1 minute was over.
- ✓ Wash it with tap water and drain (do not blot).
- ✓ Wash it with 95% ethyl alcohol for 30 seconds.

- ✓ Wash it with tap water at the end of 30 seconds to stop decolorization and drain.
- ✓ Counter stain with 0.25% saffranin for 30 seconds.
- ✓ Washing is again done followed by draining and examining under microscope at 100x objective.

Biochemical Tests
Indole Test
Principle

The Indole test is used to assess the ability of an organism to divide the compound indole with the amino acid tryptophan. To generate three potential end products, Tryptophan is hydrolyzed by tryptophanase, one of which is indole. Kovac 's reagent or Ehrlich's reagent containing 4(p)-dimethylamine-benzaldehyde detects the development of indole; this reacts with indole to form a red coloured compound. Enterobacteriaceae and other genera help to be distinguished. Indole is provided by tryptophan reductive deamination [9]. The reaction's final products are indole, pyruvic acid, ammonium and energy. The need for pyridoxal phosphate as a coenzyme.

Procedure

- ❖ Inoculated the tryptophan broth with broth culture or emulsify isolated colony of the test organism in tryptophan broth.
- ❖ Incubate at 37 °C for 24-28 hours in ambient air.
- ❖ Add 0.5 ml of Kovac's reagent to the broth culture.

Sugar Fermentation Test
Principle

The sugar fermentation test is used to-

A) Find the ability of microorganisms to ferment the given carbohydrate.

B) Determine the ability to degrade amino acids.

C) Determine the ability of microorganisms to produce gaseous end products in fermentation.

The test is used to determine whether a particular carbohydrate can be fermented by a bacterium or not. In the medium, there is a pH indicator such as bromocresol purple (BCP), bromothymol blue (BTB) or phenol red that can detect a reduction in the pH of the medium due to acid production. Depending upon the organisms involved and substrate being fermented, the end products may vary. Lactic acid, formic acid, acetic acid, butyric acid, butyl alcohol, acetone, ethyl alcohol, CO_2 and hydrogen are typical end products of bacterial fermentation. Color change is only observed when sufficient amounts of acid are produced [10].

Procedure

- ❖ Prepare broth media by mixing all ingredients in distilled water and heat gently to dissolve it (add specific carbohydrate source based on the test requirement, one at a time).
- ❖ Maintain the pH at 7.4
- ❖ Autoclave the prepared test media to sterilize.
- ❖ Aseptically, inoculate the test tube with the test microorganism using an inoculating loop or needle.
- ❖ Incubate tubes at 37ºC for 18-24 hours.

MR-VP Test
Principle

The Methyl Red (MR) test decides whether or not the microbe undergoes mixed acid fermentation when glucose is supplied. One of the main taxonomic characteristics that help distinguish different genera of enteric bacteria is the types and proportions of fermentation products formed by anaerobic fermentation of glucose. Acid products (mainly lactic or acetic acid), ethanol, carbon dioxide and hydrogen are produced by the pathway. These large amounts of acid results in significant decrease in pH of the medium below 4.4.

To assess whether an organism develops acetyl-methyl carbinol from glucose fermentation, the Voges-Proskauer test is used. The VP test recognises species that use the pathway of butylene-glycol to generate acetoin. The end result of acetoin is oxidised into diacetyl in the presence of potassium hydroxide. Diacetyl then reacts to red colour development [11]. Creatine is also present in the reagent as a catalyst. The MR-VP reagents are useful in the identification of Enterobacteriaceae.

Procedure

- ❖ MR-VP broth is being prepared and autoclaved to sterilize.
- ❖ The broth is left to cool down for a while.

- ❖ Inoculation is being done with a pure culture of the microorganisms under investigation.
- ❖ The broth is incubated at 37 ℃ for upto 48 hours.
- ❖ Add about 5 drops of the methyl red indicator solution to the first tube.
- ❖ Add about 600ul of VP-reagent A and about 400ul of VP-reagent B to the second test tube.

Urease Test

Principle

The urease test was used to determine an organism's ability to break the urea by generating the enzyme urease. Urea is the result of amino acid decarboxylation. Ammonia and carbon dioxide are formed by hydrolysis of urea. The formation of ammonia alkalinizes the medium and the colour change of phenol red from light orange at pH 6.8 to magnet (pink) at pH 8.1 is observed by the pH change. Urea is a diamide of carbonic acid. *Proteus species, Cryptococcus species, Corynebacterium species, Helicobacter pylori* etc. are some of the urease positive organisms [12].

Procedure

- The broth medium was being prepared and the pH is maintained at 6.8
- Next, the broth was autoclaved and left to cool down for a while.
- Inoculation is done with a loopful of pure culture of the test organism.
- Incubate the test-tube at 37 ºC in ambient air for 24 hours and predict the results accordingly.

Catalase Test

Principle

Catalase was an enzyme which is produced by microorganisms that live in oxygenated environment to neutralize toxic forms of oxygen metabolites, H_2O_2. Anaerobes generally lack the Catalase enzyme.

The breaking down of hydrogen peroxide into oxygen and water is regulated by catalase. To assess whether a bacterial isolate is capable of producing the Catalase enzyme, a small inoculum of bacterial isolate is mixed into a solution of hydrogen peroxide and observed for rapid oxygen bubble processing. Strict aerobes as well as optional anaerobes contain catalase positive bacteria [13]. They all have the ability to respire oxygen as a terminal electron acceptor.

Procedure

- Prepare nutrient agar media and autoclave it.
- Transfer a small amount of bacterial colony to the slant and streak it.
- Incubate the test tube at 37°C for 24 hours.
- Add 4-5 drops of 3% hydrogen peroxide to the test tube.

Citrate Test

Principle

The citrate utilisation test was used to assess the ability of bacteria to use sodium citrate as their only source of carbon and the sole fixed source of nitrogen inorganic $NH_4H_2PO_4$. Alkaline carbonates and bicarbonates are inevitably formed when an organic acid such as citrate is used as a carbon and energy source. The use of exogenous citrate allows citrate transport proteins (permeases) to be present. Citrate is divided by citrate lyase into oxaloacetate and acetate upon uptake by the cells. Pyruvate and CO_2 are then metabolised into oxaloacetate [14)]. Growth usually results in the Bromothymol blue indicator, turning from green to blue. Examples of citrate positive organisms include *Klebsiella pneumonia*, *Enterobacter species*, and *Citrobacter freundii*, *Providencia*, *Salmonella* (other than *typhi* and *paratyphi* A) etc.

Procedure

- Prepare the citrate agar media and maintain the pH up to 5.
- Autoclave for sterilization purpose.
- Shake constantly until cooling and keep it in a slant manner.
- Inoculate lightly on the slant by touching the tip of the needle.
- Incubate at 37 °C for up to 18-24 hours.

Starch Test

Principle

The goal of the starch test is to see whether starch, a complex carbohydrate made from glucose, can be used by the microbe as a source of carbon and energy for development. Starch use is achieved by an enzyme called alpha-amylase. Using a medium containing starch.

The iodine reagent is applied after inoculation and incubation to detect the presence of starch. To form a blue-black colour in the culture medium, iodine reagent complexes with starch. Because of the presence of alpha amylase, clear halos around colonies suggest their ability to digest the starch in the medium [15].

Procedure

- Prepare the media by mixing all the required composition.
- Autoclave the media and let it cool down till solidification is done.
- Use a sterile inoculation loop to pick up the culture and perform streaking.
- Place the inoculated plate into the incubator at 370C for at least 24 hours.
- Retrive the incubated culture and add iodine solution with the help of pipette on the media.

Casein Hydrolysis Test
Principle

To decide whether an organism can generate the exoenzyme casease, the casein hydrolysis test is performed. In order to degrade casein, casease is an exoenzyme that is formed by certain bacteria. Casein is a large protein responsible for milk's white colour. Protein casein is a large amino acid polymer which makes up about 85% of the protein found in milk. Casein is way too large to enter the cell membrane. In order to utilize casein, bacterial cells secrete proteolytic enzymes outside of the cell that hydrolyze the protein in steps to amino acids [16].

After crossing the cell membrane through transport proteins, the amino acids can then be used by the cells. The test is useful in recognising milk-growing bacteria and differentiating between Enterobacteriaceae, Bacillaceae and several other families.

Procedure

- Prepare the media by mixing all the contents (except casein) and autoclave it for the purpose of sterilization.
- Add casein and shake constantly to prevent clump formation.
- Perform inoculation with the inoculation loop and streak it on the media plate.
- Incubate the plates for 24 hours at 37 °C.

Minimum Inhibitory Concentration (MIC):
Principle

In microbiology, the minimum inhibitory concentration (MIC) is the lowest concentration of a chemical which prevents visible growth of a bacterium (in other words, at which it has bacteriostatic activity).

This varies from the minimum bactericidal concentration (MBC), which is the concentration that results in the death of microbes. The concentration at which it is bactericidal [17], in other words. The lowest concentration of an antibacterial agent needed to kill a specific bacterium is the minimum bactericidal concentration (MBC).

Procedure

The MIC of the chemical is determined by preparing, at increasing concentrations, solutions of the chemical in vitro, incubating the solutions with the inoculated broth sample and calculating the effects by means of serial dilution of the broth. By using a cut-off point, findings have been categorised into susceptible (often called sensitive), moderate, or resistant to a specific antibiotic.

Cut off points are agreed upon principles, published in a reference body 's guidelines, such as the U.S. The Institute for Clinical and Laboratory Standards (CLSI), the British Society for Antimicrobial Chemotherapy (BSAC) and the European Antimicrobial Resistance Research Committee (EUCAST).

MBC can be determined from broth dilution minimum inhibitory concentration (MIC) tests by sub-culturing to agar plates that do not contain the test agent. By deciding the lowest antibacterial agent concentration that decreases the viability of the initial bacterial inoculum by about 99.9 percent, the MBC is recognised. The MBC is similar to the MIC; while the MIC test shows the lowest level of growth-inhibiting antimicrobial agent, the MBC shows the lowest level of antimicrobial agent that results in microbial death.

This implies that plating the bacteria on agar could still result in organism proliferation even though a specific MIC showed inhibition, since the antimicrobial did not cause death. If the MBC is no more than four times the MIC, antibacterial agents are commonly known as bactericidal.

RESULT AND DISCUSSION

Sample Collection for Extraction

Extract samples (Clove, Fennel, Asafoetida, Cumin, Ajwain) were collected from Market of Indira Nagar, Lucknow.

Isolation of Bacteria

Bacteria isolation was done by spreading the soil sample on the media and colonies of bacterial growth were obtained.

Clove **Fennel** **Asafoetida** **Cumin** **Ajwain**

Figure 1: Extract Sample of Spice

Streaking

Streaking was done by spreading plate for obtaining single colony of bacteria.

Gram Staining

In order to study morphology and shapes of bacteria, gram staining was performed. Depicts gram positive rod-shaped bacteria i.e. *Bacillus*.

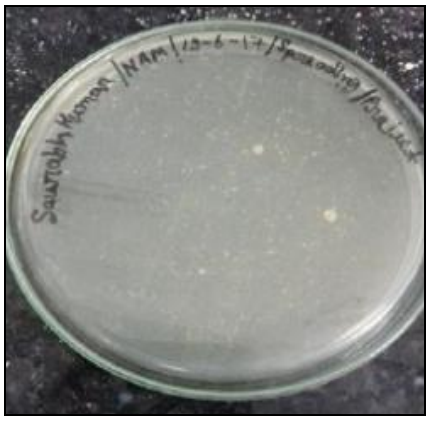


Figure 2: Isolation of Bacteria from Water Sample as Illustrated in Structure

Figure 3: Pure Culture Isolation of Sample to Streak Out Different Colonies

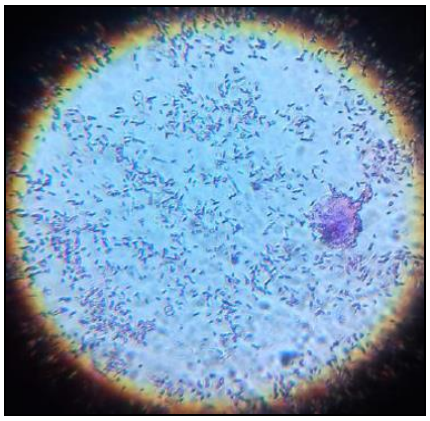


Figure 4: Gram-Staining of Bacterial Species Gram-Negative Rod-Shaped Bacteria were Visualized as They Did Not Retain the Purple Colour

Identification of Isolated Bacterial Species by Using Biochemical Tests

Indole Test

The bacterial species showed negative result for indole test which means these bacteria are not able to produce tryptophanase enzyme after addition of Kovac's reagent.

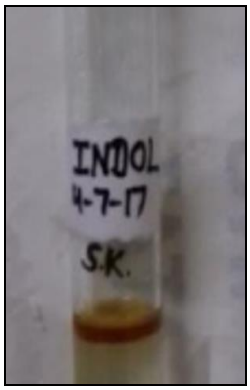


Figure 5: Figure as on Adding Kovac's Reagent there was no cherry red color Ring Formation

Sugar Fermentation Test

The sugar fermentation test depends upon the ability of the bacteria to ferment a particular sugar which is depicted by the color change from red to yellowish orange after a period of incubation.

Figure 6: (a) and (b) Depict a Positive Sugar Fermentation Test for Glucose and Maltoseas there is a Color Change from Red to Yellowish-Orange. Figure (c) Depicts Negative Sugar Fermentation Test for Lactose for Bacterial Species (A)

MR-VP Test

The results were determined by the cherry red color formation on addition of methyl-red reagent to the test tube for MR test and reagents VP-1 and VP-11 to the test test for VP test respectively.

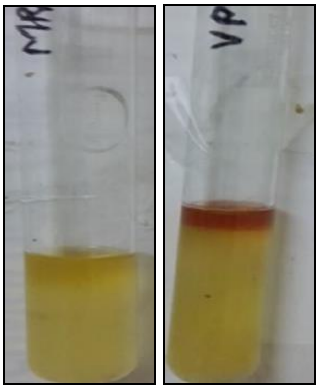


Figure 7: (a) and (b) Depict a Negative MR test for Both Bacterial Species as There is no Cherry Red Color Ring Formation

Urease Test

The result for the urease test was determined by the appearance of a pink color ring at the top of the broth after incubation of 24 hours.

Catalase Test

The result for the Catalase test is determined by the appearance of bubbles after the addition of hydrogen peroxide on the slant after the period of incubation.

Figure 8: A Positive Urease Test for Bacteria a Due to the Appearance of Pink Color Ring at the Top

Figure 9: Figure Demonstrates a Positive Catalase Test for Bacteria a as There is Appearance of Bubbles on Addition of H_2O_2

Citrate Test

A positive citrate test is reported when there is a change in color from green to blue of the incubated slant or there is appearance of microbial growth on the slant.

Figure 10: The Figures Determine a Positive Citrate Test for Both Bacterial Species as There is Microbial Growth being Visualized on the Slant which is Clearly

Casein Test

Casein hydrolysis test is supposed to be positive when there is appearance of a clear zone around the streak on the incubated plate.

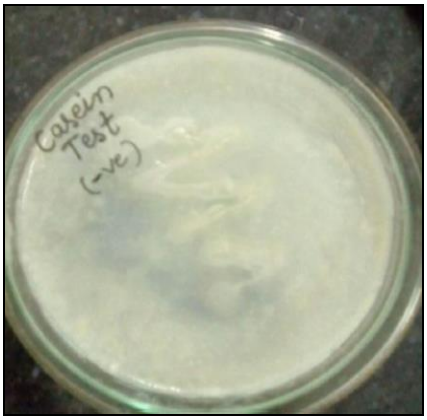


Figure 11: Both the Figures Depict a Negative Casein Test for Both Bacterial Species as There is no Clear Zone Visualized Around the Streak

Identification of Bacterial Species by using Bergey's Manual

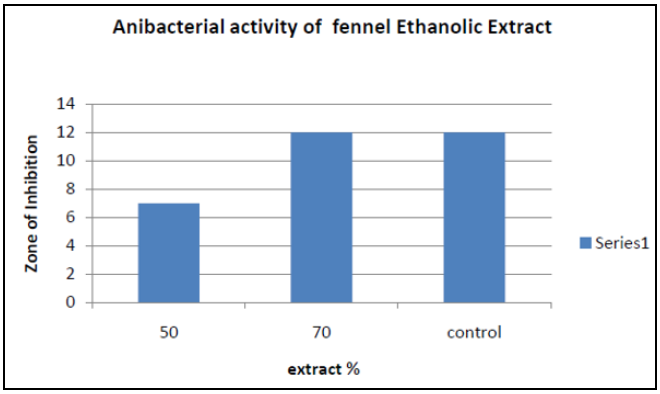
Table 1: Biochemical Test for Identification of Isolated Bacterial Species from Soil Sample. The Result of Biochemical Tests

S. No.	Test Name		B-A	B-B
1	Indole test		Negative	Negative
2	Sugar Fermentation Test	Glucose	Positive	Negative
		Maltose	Positive	Positive
		Lactose	Negative	Positive
3	MR test		Negative	Negative
4	VP test		Positive	Positive
5	Urease test		Positive	Negative
6	Catalase test		Positive	Negative
7	Citrate test		Positive	Positive
8	Starch test		Positive	Positive
9	Casein test		Negative	Negative

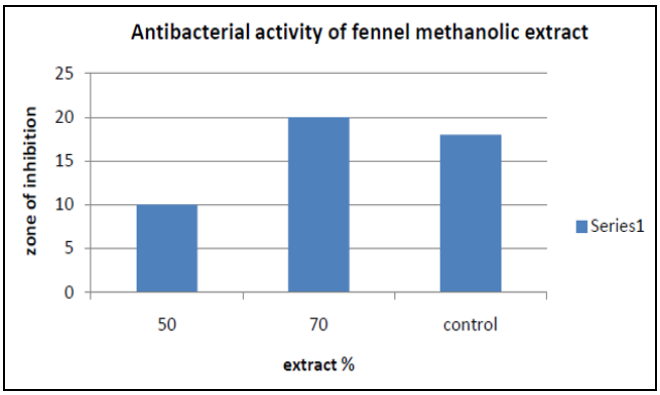
Antibiotic Test Using Agar Well Diffusion Method

Table 2: Fennel (50% and 70%)

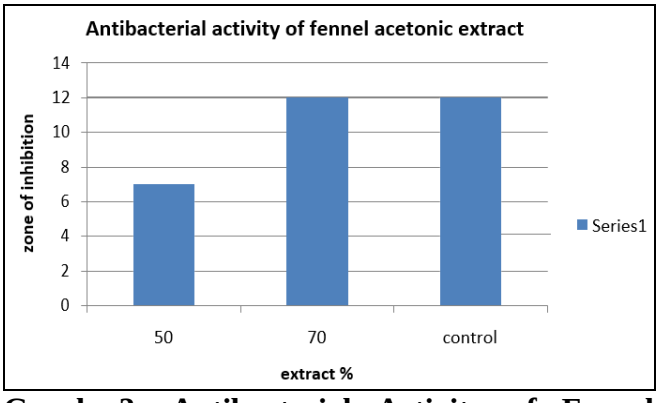
S. No.	Extract		Zone of Inhibition (mm)	Positive Control (mm)
1	Ethanol	50%	18	8
		70%	12	
2	Methanol	50%	18	7
		70%	16	
3	Acetone	50%	12	9
		70%	14	



Graph 1: Antibacterial Activity of Fennel Ethanolic Extract



Graph 2: Antibacterial Activity of Fennel Methanolic Extract



Graph 3: Antibacterial Activity of Fennel Acetone Extract

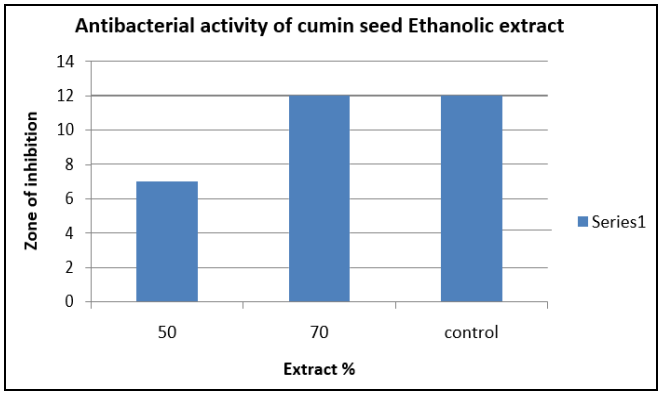
Figure 12: Antibacterial Activity of Fennel (A) Ethanolic Extract (B) Methanolic Extract (C) Acetone Extract

Table 3: Cumin Seed- (50% And 70%)

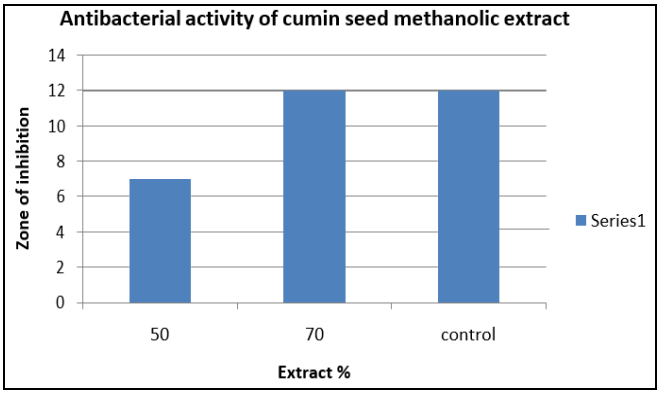
S. No.	Extract		Zone of Inhibition (mm)	Positive Control (mm)
1	Ethanol	50%	32	6
		70%	31	
2	Methanol	50%	12	8
		70%	12	
3	Acetone	50%	15	7
		70%	14	

Table 4: Carom Seed-(50% and 70%)

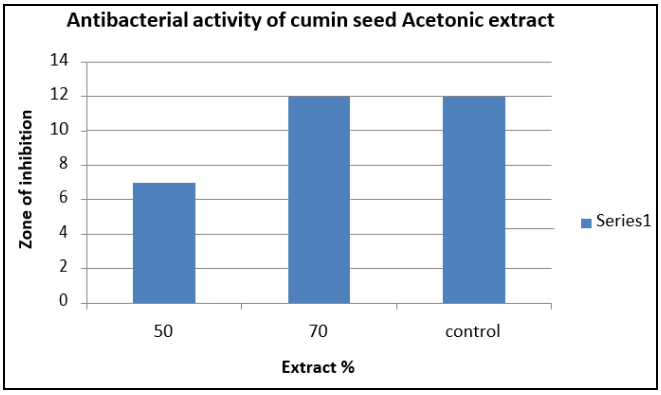
S. No.	Extract		Zone of Inhibition (mm)	Positive Control (mm)
1	Ethanol	50%	20	10
		70%	18	
2	Methanol	50%	20	7
		70%	18	
3	Acetone	50%	30	9
		70%	28	



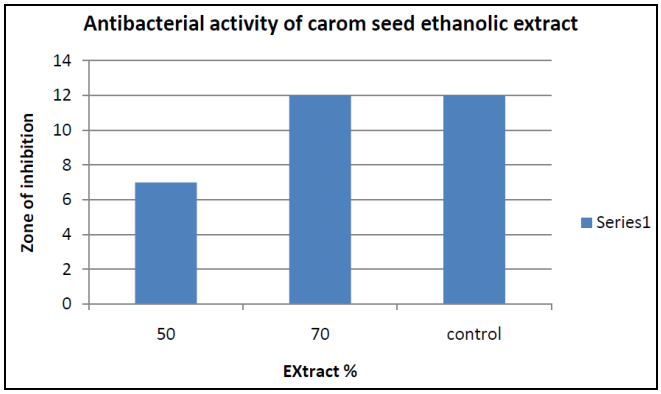
Graph 4: Antibacterial Activity of Cumin Seed Ethanolic Extract



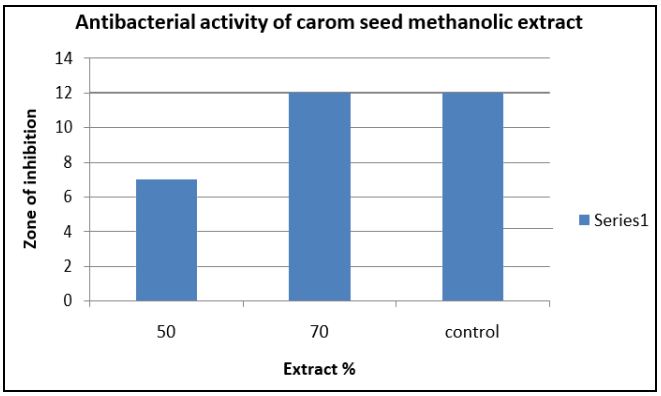
Graph 5: Antibacterial Activity of Cumin Seed Methanolic Extract



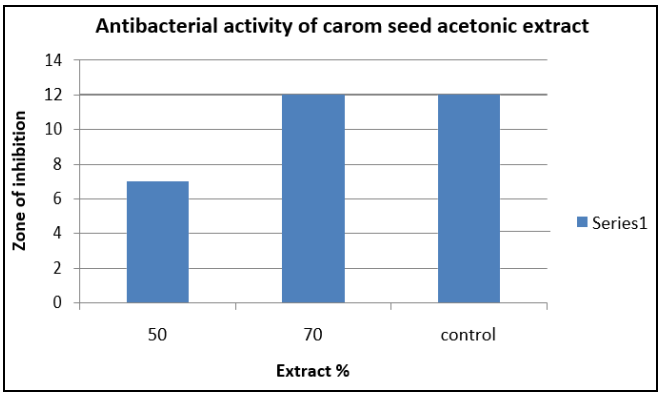
Graph 6: Antibacterial Activity of Cumin Seed Acetone Extract



Graph 7: antibacterial Activity of Carom Seed Ethanolic Extract



Graph 8: antibacterial Activity of Carom Seed Methanolic Extract



Graph 9: Antibacterial Activity of Carom Seed Acetonic Extract

Figure 13: Antibacterial Activity of Cumin Seed (A) Ethanolic Extract (B) Methanolic Extract (C) Acetone Extract

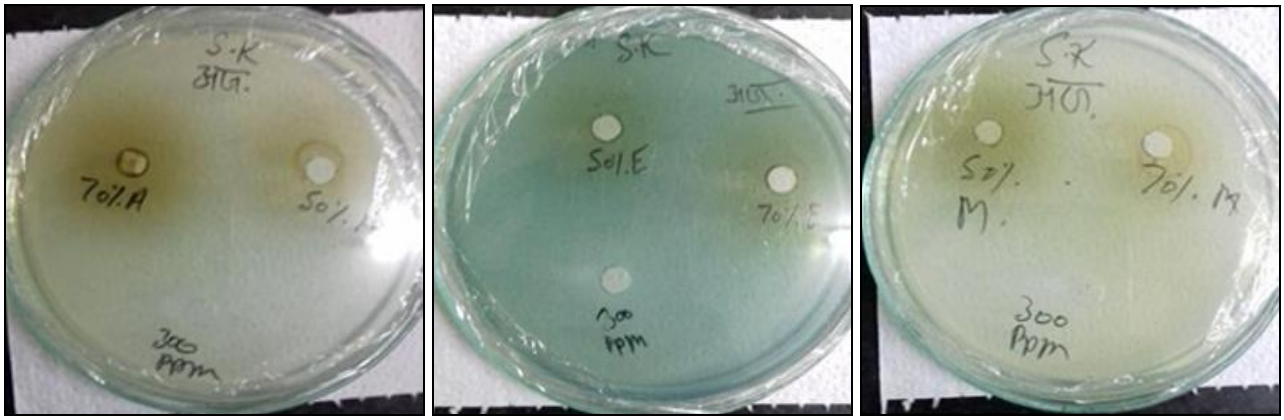
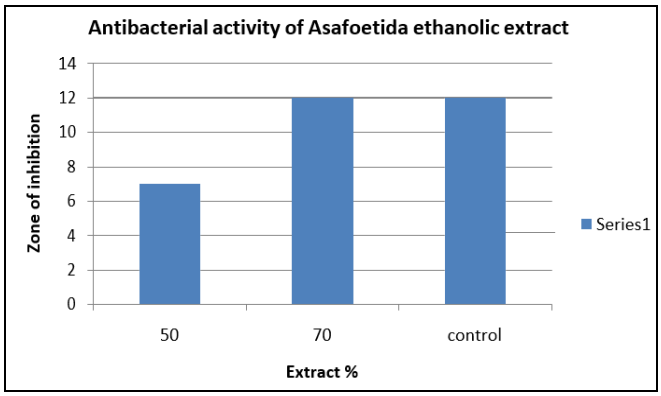


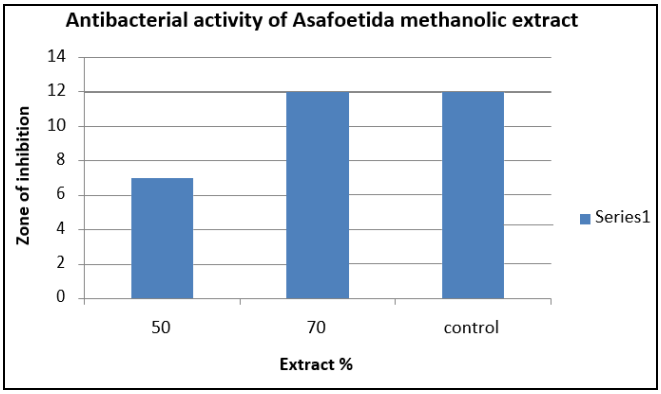
Figure 14: Antibacterial Activity of Carom Seed (A) Ethanolic Extract (B) Methanolic Extract (C) Acetone Extract

Table 5: Asafoetida- (50% and 70%)

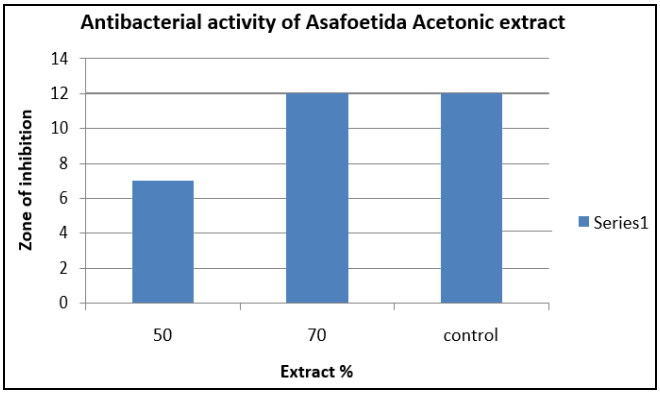
S. No.	Extract		Zone of Inhibition (mm)	Positive Control (mm)
1	Ethanol	50%	14	6
		70%	18	
2	Methanol	50%	13	9
		70%	10	
3	Acetone	50%	12	7
		70%	12	



Graph 10: Antibacterial Activity of Asafoetida Ethanolic Extract



Graph 11: Antibacterial Activity of Asafoetida Methanolic Extract



Graph 12: Antibacterial Activity of Asafoetida Acetonic Extract

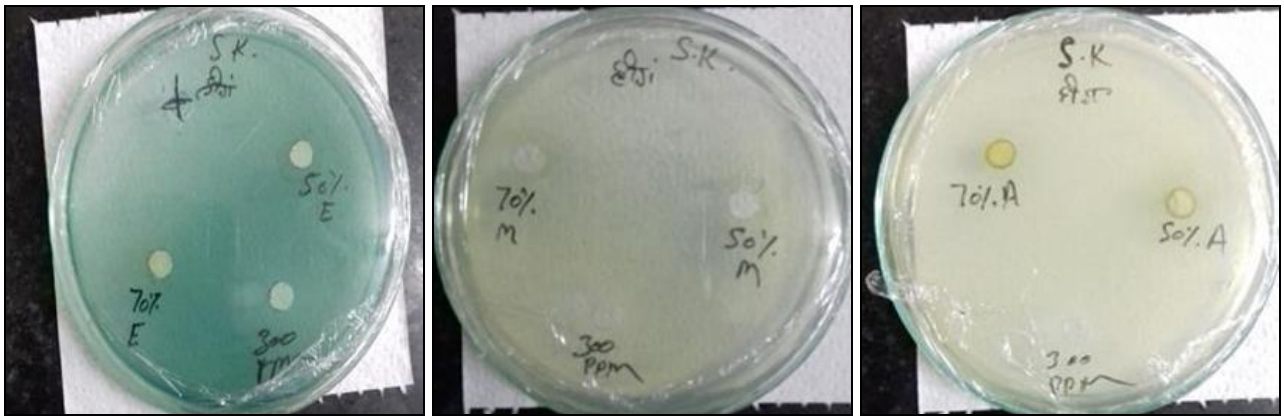
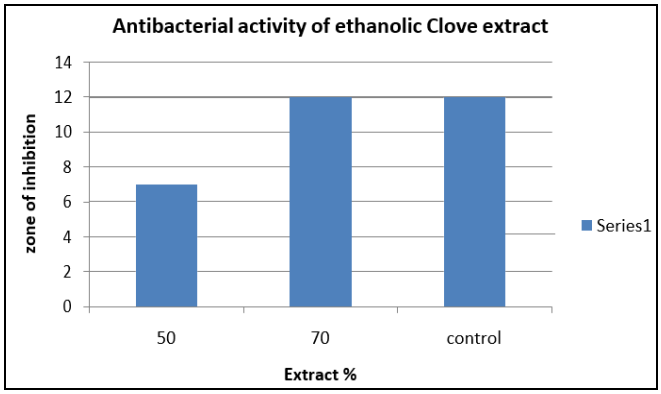


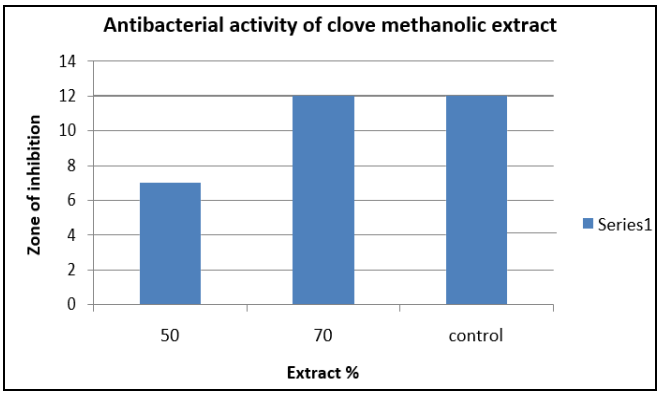
Figure 15: Antibacterial Activity of Asafoetida(A) Ethanolic Extract (B) Methanolic Extract (C) Acetone Extract

Table 6: Clove-(50% And 70%)

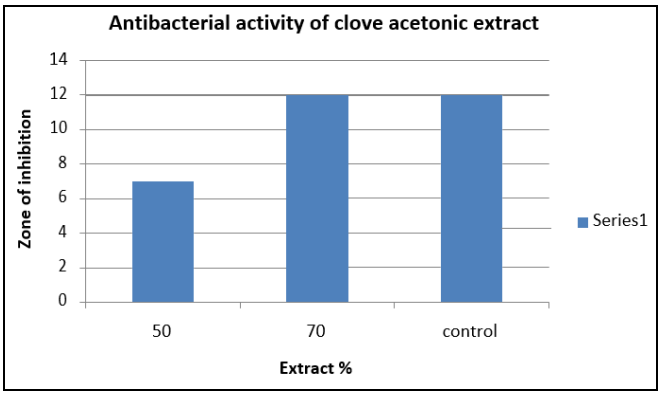
S. No.	Extract		Zone of Inhibition (mm)	Positive Control (mm)
1	Ethanol	50%	18	8
		70%	17	
2	Methanol	50%	19	9
		70%	18	
3	Acetone	50%	28	12
		70%	29	



Graph 13: Antibacterial Activity of Clove Ethanolic Extract



Graph 14: Antibacterial Activity of Clove Methanolic Extract



Graph 15: Antibacterial Activity of Clove Acetonic Extract

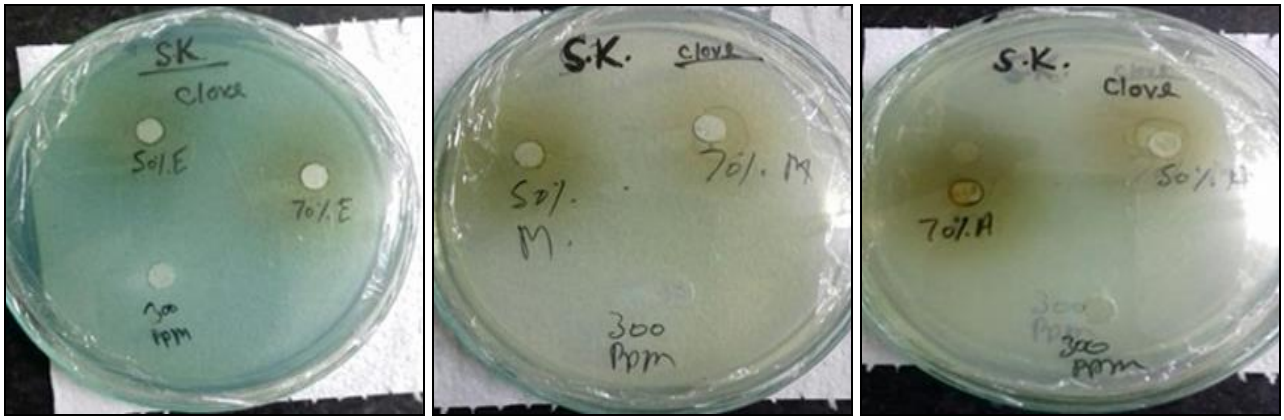


Figure 16: Antibacterial Activity of Clove (A) Ethanolic (B) Methanolic (C) Acetone Extract

The process of agar well diffusion is ultimately carried out to assess the desired sample 's antibacterial or antibiotic activity. When a clearance zone is visible around the well, the result is expected to be positive, and if no clearance zone is obtained, it is a negative result. The clear zone defines the duration of the inhibition zone in which no bacterial growth takes place.

Minimum Inhibitory Concentration (MIC) Test

The extract's MIC value was calculated as the lowest concentration that, after 48 hours of incubation at 37 °C, completely inhibited bacterial growth. MIC was observed at 10-2 dilution for the given extract. As growth was observed after this concentration and turbidity was seen in the media.

Figure 17: The Above Picture Depicts Serial Dilution for MIC

CONCLUSION

Not only are spices renowned for their exquisite taste and fragrance, they are also abundant in antioxidants and antimicrobial properties and are thus extremely beneficial to human health. The goal of the project is to concentrate on spices as antibacterial agents. Secondary metabolites of spices like volatile oil of Fennel (*Foeniculum vulgare*), extracted oil of cloves (*Syzigium aromaticum*), Asafoetida (*Ferula foetida*), and extracts of carom (*Cinnamomum zeylanicum*) possess antibacterial properties in varying concentrations. The project describes the methodologies we have carried out to test the antimicrobial (antibacterial) properties of spices against the species of Vibrio, namely, clove, ajwain, Asafoetida, cumin and fennel. To achieve optimum effectiveness, the antimicrobial properties of spices at different concentrations are carried out. Methanol and acetone extract of 5 Indian spices were screened for antibacterial properties in search of a bioactive compound. MIC was observed at 10^{-2} dilution for the given extract. As growth was observed after this concentration and media showed turbidity.

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CONFLICT OF INTEREST

None

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