International Journal of Scientific Research in Engineering and Management (IJSREM)
Volume: 08 Issue: 07 | July - 2024 SJIF Rating: 8.448 ISSN: 2582-3930

Identification of Dominant Bacteria from Crowded Places of Vellore by using MIC and ABST Analysis

Pothineni Yaswanth[1], R B Sai Teja[1], Tejasri Sirigiri[1], Sanhita Pathak[1], Dr. Suneetha Vuppu[1]*

[1]Department of Biotechnology, School of Bio Sciences and Technology, Vellore Institute of Technology, Vellore – 632014, India
**vsuneetha@vit.ac.in*

ABSTRACT- The purpose of this study is to understand the antimicrobial effect of spices and leaf extracts against prominent bacteria present in crowded localities. It has been shown that spices offer therapeutic benefits, particularly antimicrobial action. In addition to being food preservatives, they are employed as household medications. In this work, agar well diffusion method is used to compare the susceptibility of a few human pathogenic bacteria to different spice extracts. The extracts were prepared using 70% ethyl alcohol. The samples for the pathogenic bacteria were collected from crowded places situated in Vellore, Tamil Nadu. The antimicrobial properties of bilva, lemon leaves, mehndi, cinnamon, and hibiscus leaves were found to be comparatively higher than those of the other spices and leaf extracts examined. Of all the spices, ethanol extracts from cinnamon had the strongest antimicrobial properties. It has been discovered that Gram negative bacteria are more susceptible to leaf extracts and spices than Gram positive bacteria. The potential application of spices as antimicrobial agents is quite high.

***Key Words*:** spices, leaf extracts, antimicrobial activity, ethanol extract, MIC, ABST

1.INTRODUCTION

Herbs and spices are essential components of the human diet, recognized not only for their flavor-enhancing properties but also for their antimicrobial and therapeutic benefits. Historically, these natural additives have been used for their preservative qualities and to treat various ailments, as documented by researchers like Bagamboula et al. (2003) and Arora & Kaur (1999). With the increasing resistance of bacterial infections to antibiotics (Gold & Moellering, 1996) and the adverse effects of conventional therapies, there has been a significant shift towards herbal and traditional medicine, which the World Health Organization (WHO) notes is relied upon by over 80% of the global population for medical needs (WHO, 2002).

In recent years, the safety and efficacy of spices and herbs in treating illnesses have gained attention in contemporary science (Chaudhry & Tariq, 2006). These natural remedies are particularly prevalent in Asian, African, and other regions, and their use is growing in industrialized nations due to their health benefits. Airborne diseases, such as chickenpox, the common cold, and COVID-19, highlight the need for effective antimicrobial agents, which spices can provide through their natural properties.

The use of plant-based components in pathogen control is promising, especially considering the antibacterial qualities of spices. These natural alternatives to artificial food additives can help combat infections and ensure food quality and consumer safety (Singh et al., 2007). Despite their potential, the application of spices in this context has been somewhat limited, warranting further exploration and utilization.

Spices owe their medicinal benefits to the antibacterial action of various bioactive compounds, including flavonoids, alkaloids, tannins, phenolic compounds, coumarins, glycosides, terpenes, and isoflavonoids. These compounds not only offer health benefits but also enhance the economic value of spices, which are widely used in cosmetics, medicines, perfumes, and food products (El-Gammal, 1993; Loewenfeld & Back, 1979).

In conclusion, the rising interest in the health benefits of herbs and spices reflects a broader trend towards natural and traditional medicine. As the world grapples with antibiotic resistance and the side effects of conventional treatments, the antimicrobial properties of spices present a valuable alternative. Emphasizing their role in both diet and medicine can lead to safer, more effective health solutions globally.

2. MATERIALS AND METHODOLOGY

Section 2.1- Collection Of Plant Materials

The spices and leaves listed in Table 1 were brought from households located in *Kuppam* district (cinnamon, lemon leaves, bilva leaves) and VIT Vellore (hibiscus leaves, mehndi leaves) in freshly collected form. These materials were identified and confirmed by our faculty in the laboratory.

Table-1: List of spices and leaves used in the evaluation of antimicrobial activities

Common Name	Botanical Name	Family	Parts used	Major Components
Cinnamon (Daalchini)	*Cinnamomum zeynalicum Bl.*	*Lauraceae*	Bark	Trans-cinnamaldehyde and eugenol
Hibiscus	*Hibiscus rosa-sinensis*	*Malvaceae*	Leaves	Alkaloids, tannins, resins
Mehndi	*Lawsonia inermis*	*Lythraceae*	Leaves	p-coumaric acid, 2-methoxy-3-methyl-1,4-naphthoquinone and apiin
Bilva Leaves (Bel)	*Aegle marmelos L.*	*Rutaceae*	Leaves	Xanthotoxin, Umbelliferon
Lemon Leaves	*Citrus limon*	*Citrus*	Leaves	Sabinene, Carene, Limonene, and β-ocimene

Section 2.2- Processing of Sample

The spices were blended in a mixer. The leaves were completely dried followed by grinding to produce powder form of samples. The blended samples were collected into autoclave bags for storage. These were then used for making extracts using distilled water and ethanol successively.

Section 2.3- Extraction of Spices and Leaves Material

The spices powders were loaded into Eppendorf tubes and subjected to extraction with solvent like ethanol to obtain ethanol extracts was performed.

Section 2.4- Collection of Bacterial Samples

Petri dishes with Muller Hilton agar and McConkey agar were prepared in the laboratory for the purpose of sample collection. The sample collection was performed by Exposure Plate Technique, i. e., exposing the petri dishes to the environment around a running train in the Katpadi Railway Station. The plates were immediately sealed with parafilm after exposing to the surroundings and incubated in the laboratory.

Section 2.5- Preparation of Standard Bacterial Culture

Upon incubation of samples at room temperature for 24-48 hours 4 distinct colonies were observed. Each of these colonies were streaked onto separate plates and sub-cultured every 4 days to prevent contamination. Pure cultures were prepared by suspension method. 3 out of the 4 samples were found to be bacterial samples. Hence these 3 samples were taken for suspension preparation and further studies. A loopful of each sample was taken and inoculated into 2ml nutrient broth Eppendorf individually by smearing. They were labelled as T1, T2, and T3 (test samples), respectively.

Section 2.6- Studies performed to understand the morphology of the Microbial Isolates

The tests performed include gram staining, endospore staining, capsule staining, and oxidase test.

The results obtained are available under Table 2.

Section 2.7-Evaluation of Antibacterial Activities of Spices and Leaves Extract

The crude extracts of spices and leaves were checked for their antibacterial activity against the organisms by agar well diffusion method. The tests performed were anti-bacterial susceptibility test (ABST) and minimum inhibitory concentration (MIC) test. After dipping a sterile cotton swab into the inoculums, the swab was rotated through a 60° angle to seed the entire Muller Hilton and McConkey agar plates. The agar surface was finally cleaned by passing the swab around its borders, and the lid was closed to let the mixture dry for a few minutes at room temperature. The micropipette was used to dispense 100µl and 50µl of the working suspensions of the spice and leaves extracts into the corresponding wells. Simultaneously, the solvent's activity was assessed as a control. Following a 24-hour incubation period at 37°C, the plates verified for the zone of inhibition, indicated by the well's unobstructed vicinity.

Section 2.8-Minimum Inhibitory Concentration

The MIC test is a method used to determine the lowest concentration of an antimicrobial agent that inhibits the growth of microorganisms in-vitro. It determines the antimicrobial activity of a test agent against a specific bacterium. Methods include E-test, tube dilution, and agar dilution. To conduct the test, a sample was taken, contaminated, and serial dilution was performed. The agar was then autoclaved and swabbed on an agar plate. 20 holes were punched in each petri plate, and 0 µl to 50 µl of the working suspensions of spice and leaf extracts

were dispensed into the wells. The solvent's activity was assessed as a control. After a 24-hour incubation period at 37°C, the zones of inhibition were verified.

Section 2.9-RESULTS AND DISCUSSION

The study evaluated the antibacterial activities of various spices and leaf extracts. Cinnamon was found to be the most effective against tested organisms, with a broad spectrum of inhibitory effects. The sensitivity of Gram-positive bacteria to the spices was higher than that of Gram-negative bacteria. The spice and leaf extracts produced a significant inhibition zone when their volume increased, despite being less effective at lower volumes. Hibiscus leaf was effective against the bacteria after cinnamon, while Mehndi showed weak inhibitory activity.

Tests were conducted on bacterial samples, with only organism 2 tested positive and organisms 1 and 3 tested negative. The capsule staining method was positive for organisms 2 and 3, and negative for organism 1. The MBC of various effective fractions was displayed in Table 3. The spices had a greater effect on Gram positive bacteria than on Gram negative bacteria. However, different test settings and methodologies, as well as concentration variations, could cause contradictory results. Most essential oils have poor solubility and volatilities, which may cause issues when dilution and diffusion occur in a microbiological medium.

The essential oils of cinnamon and hibiscus leaf have outstanding antibacterial qualities. Cinnamon essential oil has the lowest MBC values when tested against gram positive bacteria. Spices may be used as a substitute for artificial additions and as treatments for specific illnesses. However, different test conditions, methodologies, and concentration variation could cause the contradictory outcome. Spices' antimicrobial action is influenced by factors such as their types, concentration and composition, microbial species, substrate composition, processing settings, and storage.

Table 2: Tests performed for studying the *morphology* of bacterial samples

Morphological Tests	Organism 1 (T1)	Organism 2 (T2)	Organism 3 (T3)
Gram Staining	(-)	(+)	(-)
Endospore Staining	(+)	(+)	(+)
Capsule Staining	(-)	(+)	(+)
Oxidase Test	(+)	(+)	(+)

Table 3: Zones formed after *MIC test* are listed as follows:

(a) T3, Plate 2

Sample	Dilution 1	Dilution 2	Dilution 3	Dilution 4	Control
1	**0.6**	0.1	0.2	0.1	0.3
2	0.2	0.4	**0.7**	0.2	0.2
3	**0.9**	0.1	0.1	0.1	-
4	0.1	**0.6**	0.3	0.2	-

(b) T2, Plate 1

Sample	Dilution 1	Dilution 2	Dilution 3	Dilution 4	Control
1	-	0.1	-	-	-
2	0.2	-	-	**0.8**	-
3	0.5	-	-	-	-
4	0.5	0.1	-	-	0.1

(c) T3, Plate 1

Sample	Dilution 1	Dilution 2	Dilution 3	Dilution 4	Control
1	-	-	-	0.1	-
2	-	0.1	-	0.1	-
3	-	-	**1.9**	-	-
4	0.2	0.3	-	-	-

(d) T2, Plate 2

Sample	Dilution 1	Dilution 2	Dilution 3	Dilution 4	Control
1	-	-	-	-	-
2	0.2	-	0.1	-	-
3	0.5	-	-	-	-
4	**0.6**	-	-	-	0.4

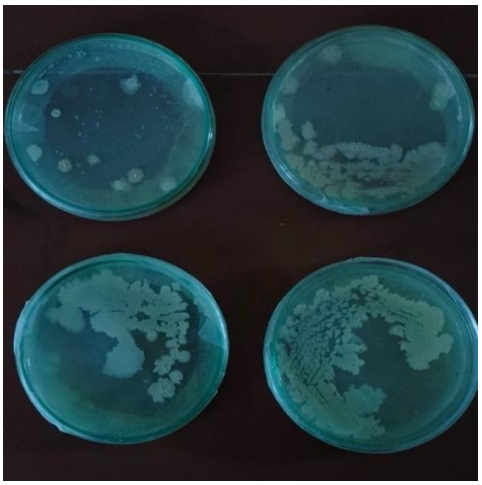

Fig -1: *Pure cultures* of bacterial strains obtained from site

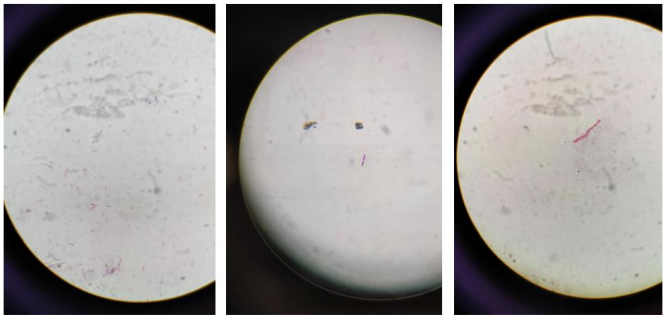

Fig -2: *Gram Staining* Results (In the order of labelling)

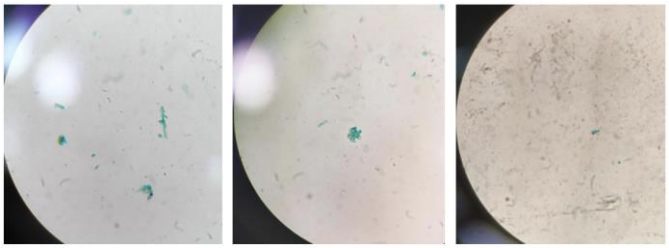

Fig -3: *Endospore Staining* Results (In the order of labelling)

Fig -4: *ABST Test* Results

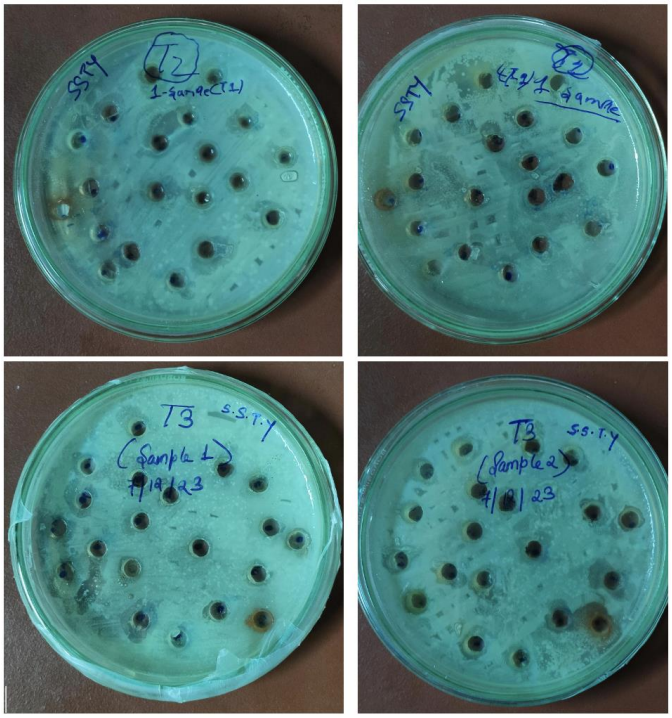

Fig -5: *MIC Test* Results (In the order of labelling)

3. CONCLUSION

In conclusion, our study successfully identified the dominant bacterial species present in crowded areas of Vellore through Minimum Inhibitory Concentration (MIC) and Antibiotic Susceptibility Testing (ABST) analyses. The results revealed a high prevalence of antibiotic-resistant bacteria, highlighting the significant public health risk posed by these environments. The identified strains exhibited varying degrees of resistance, emphasizing the need for stringent hygiene practices and robust infection control measures. Our findings underscore the importance of continuous surveillance and the development of effective strategies to combat the spread of resistant bacteria in densely populated areas.

ACKNOWLEDGEMENT

The authors would like to thank the honorable Chancellor, Dr. G. Viswanathan, Vellore Institute of Technology for his constant support and encouragement.

REFERENCES

1. Kowalska−Krochmal, B., & Dudek-Wicher, R. (2021, February 4). *The Minimum Inhibitory Concentration of Antibiotics: Methods, Interpretation, Clinical Relevance*. Pathogens. https://doi.org/10.3390/pathogens10020165
2. *Minimum Inhibitory Concentration (MIC) Test*. (2023, February 16). Swiss Anti-Bacterial & Anti-Viral Testing Laboratory, ISO Certified | MIS. https://microbe-investigations.com/minimum-inhibitory-concentration-test/
3. Aryal, S. (2022, August 10). *Capsule Staining-Principle, Reagents, Procedure and Result*. Microbiology Info.com. https://microbiologyinfo.com/capsule-staining-principle-reagents-procedure-and-result/
4. Ahern, H. (n.d.). *Differential Staining Techniques*. Pressbooks. https://milnepublishing.geneseo.edu/suny-microbiology-lab/chapter/differential-staining-techniques/
5. *Microscopy and Staining: Gram, Capsule, and Endospore Staining*. (n.d.). JoVE. https://www.jove.com/v/10513/microscopy-and-staining-techniques-in-bacteria#:~:text=Gram%2Dpositive%20bacteria%20stain%20purple,is%20added%20(Figure%203).
6. Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015, September 7). *Antimicrobial resistance: a global multifaceted phenomenon*. Pathogens and Global Health.

https://doi.org/10.1179/2047773215y.0000000030

7. *What Exactly is Antibiotic Resistance?* (2022, October 5). Centers for Disease Control and Prevention. https://www.cdc.gov/drugresistance/about.html#:~:text=About%20Antimicrobial%20Resistance,-Print&text=Antimicrobial%20resistance%20happens%20when%20germs,and%20sometimes%20impossible%2C%20to%20treat.
8. Bayot, M. L. (2022, October 10). *Antimicrobial Susceptibility Testing*. StatPearls - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK539714/
9. *Antimicrobial Susceptibility Testing - Microbiology Resource Center - Truckee Meadows Community College*. (n.d.). https://www.tmcc.edu/microbiology-resource-center/lab-protocols/antimicrobial-susceptibility-testing
10. *Antimicrobial Susceptibility Testing (AST) : Définition, Protocol, Interpretation*. (n.d.). https://microbiologie-clinique.com/antibiotic-susceptibility-testing.html
11. L. (2022, December 24). *13.5A: Minimal Inhibitory Concentration (MIC)*. Biology LibreTexts. https://bio.libretexts.org/Bookshelves/Microbiology/Microbiology_(Boundless)/13%3A_Antimicrobial_Drugs/13.05%3A_Measuring_Drug_Susceptibility/13.5A%3A_Minimal_Inhibitory_Concentration_(MIC)