IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

A REVIEW OF IN-VITRO MODELS FOR EVALUATING THE BACTERICIDAL PROPERTIES OF HOMOEOPATHIC MEDICINES.

[1] Dr Neha Ashok Shendre, [2] Dr Ami Majmundar,

[1]Associate Professor, Department of Pathology, [2]Professor& HOD, Department of Pharmacy

[1]Department of Pathology

[1]Smt. Malini Kishore Sanghvi homoeopathic Medical College, Vadodara, India

Abstract: The rapid emergence of antimicrobial resistance has intensified the search for alternative therapeutic strategies. This review analyzes various in vitro models employed to evaluate the bactericidal properties of homoeopathic medicines. A wide range of experimental methods—including agar diffusion assays, broth dilution techniques for determining minimum inhibitory concentration (MIC), time-kill assays, biofilm models, and advanced analytical techniques such as flow cytometry have been utilized to assess antimicrobial activity against organisms such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Candida albicans*. Several homoeopathic preparations, have demonstrated inhibitory effects under laboratory conditions at different potencies. By bridging traditional principles with modern methodologies, future standardized in vitro model research can contribute significantly to the integration of homoeopathy into mainstream antimicrobial strategies.

Index Terms – In-Vitro Models, Homoeopathic medicines.

I. INTRODUCTION

The global rise of antimicrobial resistance has necessitated the exploration of alternative therapeutic approaches. Homoeopathy, a holistic system of medicine, has been gaining attention for its potential antimicrobial effects clinically. Homoeopathy is based on the principle of "similia similibus curentur" (like cures like), wherein substances that cause symptoms in healthy individuals are used to treat similar symptoms in sick individuals. Homoeopathic medicines, often prepared in ultra-diluted forms, are postulated to exert antimicrobial properties via unique mechanisms. Despite skepticism regarding their efficacy due to extreme dilutions, certain homoeopathic preparations have demonstrated bactericidal and bacteriostatic effects in laboratory settings. Evaluating these properties requires robust and reproducible in vitro models, which offer controlled environments for testing. These models enable detailed observations of the interactions between the medicines and microbial cells, assessment of dose-response relationships, and insights into underlying mechanisms. This article aims to review the in vitro models used to evaluate the bactericidal properties of homoeopathic medicines, highlighting their methodologies, findings, and relevance. For homoeopathic medicines, such studies provide scientific validation and bridge the gap between clinical observations and mechanistic evidence.

I. REVIEW OF LITERATURE

Numerous studies have explored the antimicrobial potential of homoeopathic medicines using various in vitro models:

1. **Agar Diffusion Assays**:
   - Studies using agar diffusion methods, such as the Kirby-Bauer disk diffusion technique, have demonstrated zones of inhibition around disks impregnated with homoeopathic medicines. Medicines like *Echinacea*, *Belladonna*, and *Arsenicum album* have shown activity against bacteria such as *Escherichia coli* and *Staphylococcus aureus*.
2. **Broth Dilution Methods**:
   - Broth microdilution and macrodilution methods have been employed to determine minimum inhibitory concentrations (MICs) of homoeopathic medicines. Some studies have reported significant growth inhibition of bacterial pathogens at specific dilutions.

3. **Time-Kill Assays**:
   - These models assess bactericidal activity over time. Homoeopathic medicines such as *Calendula* have demonstrated time-dependent killing effects on pathogens like *Pseudomonas aeruginosa*.
4. **Biofilm Models**:
   - Homoeopathic remedies have been evaluated for their ability to disrupt biofilms. For instance, *Hepar sulphuris calcareum* and *Silicea* have shown potential in inhibiting biofilm formation in *Candida albicans* and bacterial pathogens.
5. **Advanced Techniques**:
   - Methods such as flow cytometry and scanning electron microscopy have been used to study morphological changes in bacterial cells exposed to homoeopathic medicines, suggesting structural damage or disruption of cellular functions.

Important studies on antimicrobial property of homoeopathic medicines with various invitro models are as follows:

Bacteria	Procured from	Remedy	Potency	Method	Culture media	Observation/Result	Reference
Staphylococcus aureus	Clinical isolates, maintained on MHA slants in refrigerator	Staphylococcinum Lachesis, Echinacea	30C, 200C 1M 6C, 12C 30C, 200C 1M	96 well microtiter plate assay Measured by ELISA reader BIORAD	Mueller Hinton broth	Statistically significant result (p-value <0.05 obtained for all potency of Staphylococcinum nosode, Lachesis 12C potency	1
staphylococcus epidermidis	National Collection of Industrial Microorganisms (NCIM)	Antimonium crudum, Arsenic album, Hepar sulphur, silicea, Kali bichromicum	6C, 12C, 30C, 200C, 1M, 10M	Agar well diffusion method, MIC assay	Nutrient Agar	zone of inhibition which ranges from 0.6 to 1.5 cm for different potencies MIC method- different potencies such as Arsenic album 6C, Antimonium crudum 200C, Hepar Sulphur 200C, Silicea 12C result seen	3, 2
Salmonella Typhi	MTCC Microbial Type Culture Collection and Gene Bank	Acidum Muriaticum	6CH, 12CH, 30CH, 200CH, 1M, 50M	agar well diffusion method, MIC Assay	Nutrient Broth	maximum growth inhibitory zone (GIZ) against S. Typhi in 6CH, 12CH and 30CH potencies. MIC against S. Typhi,6CH (1:2), 12CH (1:2) in 25 µL/mL and 30CH (1:1) in 50 µL/mL concentration	4
S. aureus (IDH-7473) and E. coli (IDH-13003)	Isolates obtained from the National Institute of Cholera and Enteric Diseases, ICMR	M. sebifera A.belladona E. globulus R. graveolens	12C, 30C Q 3C, 12C, 30C 30C 200C	broth-microdilution assay 96- well plate	MH broth	M. sebifera 30C, 12C, A. belladonna Q, E. Globulus 3C, 12C, 30 C has shown statistically significant results against S. aureus and against E. coli	5
Staphylococcus epidermidis	National Collection of Industrial Microorganisms (NCIM)	Sulphanilamide	6C, 12C, 30C, 200C, 1M	Agar well diffusion MIC assay	Nutrient Broth	12C potency showed 0.7cm and in 30C potency showed 0.8cm inhibition zone In, MIC Sulphanilamide 30C showed higher amount of	6

						dead cells present in the death phase.	
Streptococcus Mutans and Enterococcus Faecalis	ATCC	Hypericum perforatum, Arnica Montana, Echinacea Angustifolia and Calendula Officinalis	Q	Agar Disc diffusion method MIC assay	Blood agar and brain heart infusion agar	S. mutans - For all Q MIC for was 62.5 mg/mL E. faecalis-MIC Hypericum perforatum - 1 mg/mL, Arnica Montana and Echinacea angustifolia 4 mg/mL, Calendula officinalis 16 mg/mL.	7
C.albicans *N.gonorrhoeae* *Klebsiella pneumoniae* *E.coli* *Salmonella typhi*	ATCC	*C.albicans* polyvalent nosode *N.gonorrhoeae* nosode *K.pneumoniae* *E.coli* polyvalent *Salmonella typhi* polyvalent	35c, 100c 35c 35c, 100c 35c, 100c 30c, 100c	Agar disk diffusion MIC assay	Nutrient agar	*C. albicans* polyvalent nosode 35c, 100c, *N. gonorrhoeae* 35c *K.pneumoniae* 35c *E.coli* polyvalent nosode 100c *S.typhi* polyvalent nosode 30c have shown inhibitory effect on bacteria.	8
E. coli O157:H7, Salmonella typhi, Shigella dysentriae, Vibrio cholera, Staphylococcus aureus, Bacillus cereus, Micrococcus luteus	ATCC, ICDDR,B	Mercurius Corrosivus-6, Mercurius Solubilis-6, Pyrogenium-6, Hepar Sulfuris calcareum-6, Sulfur-6	6 C	Kirby Bauer method-Zone of Inhibition MIC and MBC were done by broth dilution method	Mueller Hinton broth	**Merc sol-** E. coli – O157:H7 ZOI- - 13.1±0.20/50 µl/disc Salmonella typhi 15.3 Vibrio cholera 14.33 sulfur-6 E. coli O157:H7- 12.67 Shigella dysenterae 10.33 Salmonella typhi 14.33 Vibrio cholera 9.13	9
Bacillus cereus and S. aureus	Lab. Stock culture	Ruta R. graveolens Fraction 1 Resorcinol and Coumarin, Fraction 2 Catechol and Coumarin Fraction 3 Catechol and Hydroquinone	Fraction 1,2 and 3	Disc diffusion method	Mueller-Hinton Agar (MHA)	B. cereus and S. aureus were inhibited at1.5 µg/mL concentrations for Fractions I, II, and III. The highest inhibition zone (mm) of each B. cereus and S. aureus were 23 and 22mm	10
Enterococcus faecalis	ATCC	Acid benzoicum Silicea	30C 6C	Disc Diffusion method	Sheep blood agar	Acid benzoicum has the maximum zone of inhibition 17.2 ± 0.65	11
Pseudomonas aeruginosa		Silicea, Sulphur, and Mercurius solubilis	1M, 6C, 12C	MIC assay		Maximum inhibitory activity was recorded (>90% inhibition)	12
Staphylococcus spp	Clinical isolates from laboratory	Apis mellifica, Graphites,	30C and 200C	Agar well diffusion method,	Mueller Hinton agar	No antibacterial action in agar well diffusion method	13

		Arsenicum album, Pulsatilla		Minimal Inhibitory Concentration (MIC) assay			

IV. RESULTS AND DISCUSSION

The reviewed in vitro models demonstrate a diverse array of methods for evaluating the bactericidal properties of homoeopathic medicines. The results are often heterogeneous due to variations in methodologies, dilution scales, and selection of test organisms which suggest, there is need for standardization in experimental protocols.

The observed antimicrobial effects of homoeopathic medicines may be attributed to several factors:

- **Nanoparticle Hypothesis**: Studies suggest that nanoparticles formed during potentization may interact with microbial cells and its components.
- **Dynamic Interaction**: Homoeopathic preparations might influence microbial metabolic pathways or cell wall integrity.

In vitro models provide a valuable framework for investigating the bactericidal properties of homoeopathic medicines. While existing studies highlight their potential, the field requires more rigorous, standardized, reproducible research and further exploration through in vivo to overcome skepticism and provide a scientific basis for their use. By bridging traditional principles with modern methodologies, future standardized in vitro model research can contribute significantly to the integration of homoeopathy into mainstream antimicrobial strategies.

II. ACKNOWLEDGMENT

The authors declare that no specific funding was received for this work. The preparation of this review article was supported by institutional academic resources

.

REFERENCES

1. Sinha N, Jadhav AB. Invitro study of antimicrobial activity of homoeopathic preparations against staphylococcus aureus. Int J Health Sci Res. 2020; 10(3):172-175.
2. Aditya G, Nadgauda SS, Rajopadhye BD. Antimicrobial Activity of Homoepathic Medicine Silicea Terra: A Narrative Review. Journal of Nonlinear Analysis and Optimization. 2023;14(01).
3. Pasalkar AD, Kathade SA, Jadhav AB et.al. Study the anti-bacterial activity of homoeopathic medicines against staphylococcus epidermidis in vitro. Int J Health Sci Res. 2019; 9(12):49-53.
4. Umashankar C, Kunchiram BN, Shinde CH. In vitro evaluation of homoeopathic medicine Acidum Muriaticum in selected potencies for its antimicrobial activity against Salmonella Typhi. Malaysian Journal of Microbiology. 2024 Feb 1;20(1).
5. Kumaravel V, Mondal M, Aggarwal M, Shaw N, Singh S, Bhattacharya S, et al. In vitro antimicrobial activity of nine homoeopathic preparations in different volumes against Staphylococcus aureus and Escherichia coli. J Intgr Stand Homoeopathy 2023;6:3-8.
6. Sarkar T, Anand PK, Jadhav AB et.al. Anti-bacterial activity of homoeopathic medicine sulphanilamide against staphylococcus epidermidis in vitro. Int J Health Sci Res. 2019; 9(12):44-48.
7. Yalgi VS, BHAT KG. Antibacterial Activity of Homoeopathic Tinctures on Bacterial Strains of Streptococcus Mutans and Enterococcus Faecalis: An In vitro Study. Journal of Clinical & Diagnostic Research. 2019 Nov 1;13(11).
8. *Munshi R, Talele G, Shah R. In-vitro evaluation of antimicrobial activities of Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Neisseria gonorrhoeae, and Candida albicans nosodes. Homeopathy. 2022 Feb;111(01):042-8.*
9. Rahim MA. Antimicrobial activity and bio-potency of homoeopathy medicines against enteric pathogens (Doctoral dissertation, University of Dhaka).
10. SAEED YS, ALI JF, MOHAMMED MA. Chemical composition, antioxidant, and antibacterial activity of Ruta graveolens (Rutaceae). Biodiversitas Journal of Biological Diversity. 2023 Jul 1;24(6).
11. Dutta, Smita Durga; Maria, Rahul Devenderlal1,. Homeopathic consideration for resistant endodontic bacteria Enterococcus faecalis: An in vitro comparative disc diffusion study. Journal of Conservative Dentistry 23(5):p 528-532, Sep–Oct 2020. | DOI: 10.4103/JCD.JCD_515_20
12. Tak DS, Jadhav RK, Gajendragadkar PR. An in-vitro study on antibacterial activity of homoeopathic medicines-Silicea, Sulphur and Mercurius solubilis against Pseudomonas aeruginosa. International Journal of Pharmaceutical Research. 2020 Apr;12(3):643-8.
13. Kabir MI, Ikram A, Sikder MB, Tushar MH, Munshi SK. Determination of the inhibitory effects of commercially available homeopathic drugs on pathogenic bacterial growth. SVOA Microbiology. 2021;2(1):1-6.