

Original Research Article

## **An *in-vitro* Study to Evaluate the Anti-Bacterial Activity of *Rauvolfia serpentina* against *Escherichia coli***

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### **ABSTRACT**

World Health Organization's recent Global Antimicrobial Surveillance System (GLASS) reveals widespread occurrence of antibiotic resistance, among which the most commonly reported resistant bacteria, is *Escherichia coli*. Due to the unethical use of antibiotics, apart from drug resistance, generation of new mutant forms of non-pathogenic bacteria is also posing a severe threat to medical science nowadays. *E. coli* is a gram-negative, facultatively anaerobic bacterium which is commonly found in the intestine. This bacterium can cause various diseases such as Urinary Tract Infection (uropathogenic *E. coli*), Gastroenteritis, and Neonatal meningitis. In rare instances, they are also responsible for Septicaemia, Mastitis, Haemolytic-Uremic Syndrome (HUS) and Gram-negative Pneumonia. This study is aimed to evaluate the antibacterial activities of homoeopathic medicine *Rauvolfia serpentina* against *E. coli*. Thus it is used in various potencies to screen the bacteria by Agar Well-Diffusion assay, MIC assay and Bactericidal study. *Rauvolfia serpentina* 6C (0.9±0.1) c.m., 30C (0.6±0.1) c.m., 200C (0.7±0.1) c.m., 1M (0.8±0.1) c.m. showed inhibitory activity against *E. coli* by Agar well-diffusion assay. *Rauvolfia serpentina* in 6C showed maximum Growth inhibitory zone (GIZ) against *E. coli* in optical density value at 600 nm by MIC assay after comparison with Positive control (Amikacin), Vehicle control (Dispensing alcohol or ethanol 90%), Culture control (broth + culture), Negative control (Mueller Hinton Broth). Also in Bactericidal study with *Rauvolfia serpentina* 6C, maximum dead cells have been seen as compare to the live cells in the death phase.

**Keywords:** Anti-bacterial activity, *Escherichia coli*, Minimum Inhibitory Concentration, Bacteriolytic study, *Rauvolfia serpentina*, Homoeopathy.

### **INTRODUCTION**

The breakthrough invention of the antibiotic Penicillin by Alexander Fleming in 1928, the mortality rate due to infectious diseases remarkably came down. Since then,

lots of antibiotics are proved to have its therapeutic potential in treating the pathogenic manifestations. But amidst of all this, due to unethical use of antibiotics, the drug resistance to human pathogenic

bacteria has been reported from every corner of the world and it has become a serious threat for medical science nowadays. [1] A recent increase in publications on antimicrobial activity from microbes isolated from various sources indicating the importance of finding newer antimicrobials. [2-4] According to the World Health Organization, Homoeopathy is the fastest growing and second most widely accepted and used System of Medicine in the world. [5] Therefore, there is a great scope of using Homoeopathic medicine in combating this situation. Rauvolfia serpentina is commonly known as Indian snakeroot or devil pepper, *Sarpagandha* in Hindi which belongs to the Apocynaceae family has widely distributed in India mostly sub-Himalayan tracts, lower ranges of the Eastern and Western Ghats. [6] Due to an increase in infectious diseases in the society challenges are extensively increased to overcome these pathogenic microorganisms and E.coli is the most common infectious organism.

E. coli is a gram-negative, facultatively anaerobic bacterium which is commonly found in intestine. [7] Virulent strains of E. coli can cause Urinary Tract Infection (uropathogenic E. coli), Gastroenteritis, and Neonatal meningitis by transmitting through the faeco-oral route. [1,8] In rare instances they are also responsible for Orthopaedic device-associated infection or septic arthritis, Haematogenous myositis, Septicaemia, Mastitis, Haemolytic-Uremic Syndrome (HUS) and Pneumonia: Hospital-acquired pneumonia (HAP). [1,9]

This study was aimed to evaluate anti-bacterial activities of Rauvolfia serpentina on E. coli to combat the contagious diseases caused by it; as a part of Complementary and alternative medicine.

## **MATERIALS AND METHODS**

### **Procurement of Media and chemicals**

All media procured from Hi-Media, Mumbai such as Mueller Hinton Broth, Agar-Agar type 1 were A R grade. Amikacin (AMK) free base, 98% as

Positive control was procured from Sisco Research Laboratories Pvt. Ltd., Mumbai, Dispensing alcohol (ethanol 90%) according to Homoeopathic Pharmacopoeia of India, Volume I, as Positive control and Distilled water are used as a vehicle control.

### **Procurement of culture**

The culture of E. coli (Accession no. -NCIM 2931) was procured from National Collection of Industrial Microorganisms (NCIM), Pune and maintained as per given instructions.

### **Homoeopathic medicine**

Homoeopathic medicine Rauvolfia serpentina with liquid potencies 6C, 12C, 30C, 200C and 1M were obtained from standard GMP certified Homoeopathic Medicines Manufacturer.

### **Agar Well-Diffusion Assay**

Antibacterial activity of selected Homoeopathic Medicine with its various potencies against the E. coli was determined by using the Agar well diffusion method. Suspension of E. coli culture with 0.72 A.O.D. at 600 nm was swabbed on Mueller Hinton Agar plates and kept it for incubation for 30 minutes at 37°C. After incubation Agar were punched with sterilized borer of 0.4 c.m. In each well 40 µl of different potencies of Homoeopathic Medicine, Amikacin (1mg/ml) as a positive control, Distilled water and Dispensing alcohol (ethanol 90%) were used as vehicle control. Plates were incubated at 37°C for 24 hours. Zone of inhibition around the wells was measured to screen the antibacterial activity of E. coli.

### **Minimum Inhibitory Concentration**

MIC was determined by checking the growth of pathogen after adding 500 µl of different drug dilutions in Eppendorf tubes contains 500 µl of broth and 500 µl of the pathogen. There were different tubes preparations for Positive control (Amikacin), Vehicle control (Dispensing alcohol), Culture control (broth + culture) and Negative control (Mueller Hinton Broth). Readings were taken at 0 hour, 3 hours and 24 hours to calculate minimum inhibitory concentration values.

### Bactericidal studies for E. coli

Culture was inoculated in Nutrient broth and kept it at 37°C for 24 hours. Then 1 ml of culture was added with 1 ml of homoeopathic medicine at its best potency. After that, it was kept for 24 hours for incubation. After 24 hours 10 µl of the culture and 10 µl of the Trypan Blue were

added and the culture was loaded into Neubauer's Haemocytometer then WBC chamber to calculate bactericide activity.

### Statistical Analysis

All the experiments were performed in triplicates. Mean value and standard deviation were calculated by GraphPad Software, Inc.-GraphPad Prism Version 5.

## RESULTS

### Agar Well-Diffusion Assay



Figure 1: Anti-bacterial assay of Homoeopathic Medicine and controls by Agar well diffusion method.

Homoeopathic medicine Rauvolfia serpentina (6C, 30C, 200C and 1M) showed zone of inhibition around the well. The zone of inhibition ranged from 0.63 c.m. to 0.93 c.m. [Table 1]

[Table 1]:(Zone of inhibition of homoeopathic medicine Rauvolfia serpentina and controls by Agar Well Diffusion method.)

Name of Medicine	Inhibition zone (Mean ± Standard Deviation) in c. m.
Rauvolfia serpentina 0	0.0 ± 0.0
Rauvolfia serpentina 6C	0.9 ± 0.1
Rauvolfia serpentina 12C	0.0 ± 0.0
Rauvolfia serpentina 30C	0.6 ± 0.1
Rauvolfia serpentina 200C	0.7 ± 0.1
Rauvolfia serpentina 1M	0.8 ± 0.1
Positive control (Amikacin) 1 mg/ml	2.4 ± 0.1
Dispensing alcohol (Ethanol 90%) (according to HPI, volume I)	0.0 ± 0.0
Distilled water	0.0 ± 0.0
Nil	0.0 ± 0.0

### Minimum Inhibitory Concentration

Homoeopathic medicine Rauvolfia serpentina in various potencies showed Growth inhibitory zone (GIZ) against E. coli in the Eppendorf tube dilution assay by the process of Minimum inhibitory concentration (MIC), among all Rauvolfia serpentina 6C (0.36±0.01 A) showed the

best result in comparison with other Control groups.

### Bactericidal studies for E. coli

After 24 hours the treatment with the homoeopathic medicine the growth of the culture was seen in the death phase. More dead cells (black spots) are present rather than live cells (white spots).

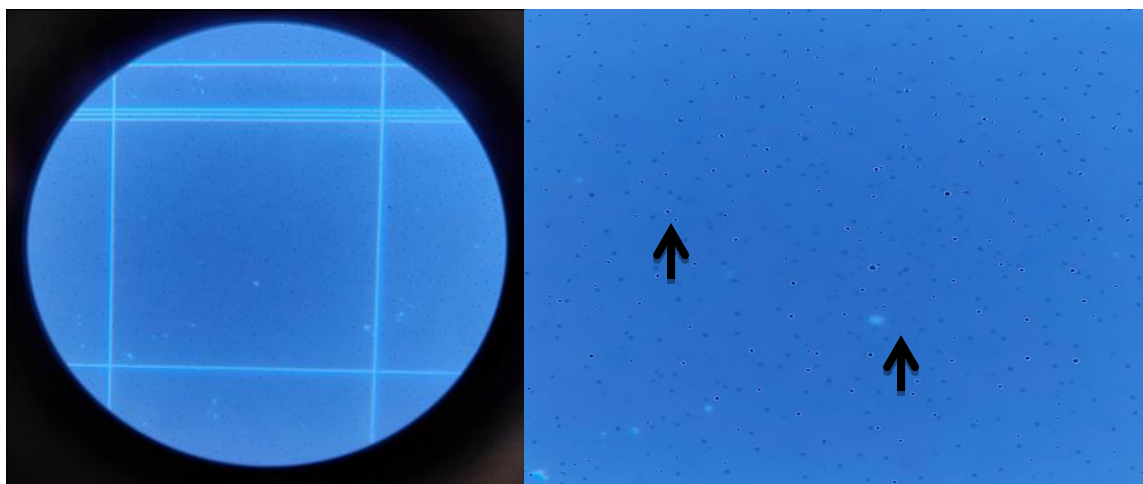


Figure 2: Microscopic view showing cell morphology by Trypan Blue stains on Neubauer's Haemocytometer.

## DISCUSSION

Recently the World Health Organization (WHO) has been developed a global priority pathogens list (global PPL) and Global Antimicrobial Surveillance System (GLASS), where global PPL contains of antibiotic-resistant bacteria by to help in prioritizing the research and development of new and effective antibiotic treatments. Here according to the species and the type of resistance the advisory board stratified the results into three (I, II, III) priority tiers: Critical, High & Medium, in which Enterobacteriaceae species (which includes E. coli) belong to the Priority I tier, i.e.; Critical. [10] Also GLASS reveals widespread occurrence of antibiotic resistance, among which the most commonly reported resistant bacteria, is E. coli. In patients, the proportion that had bacteria resistant to at least one of the most commonly used antibiotics ranged drastically between different countries, i.e.; from 8% to 65%. [11]

In combating these above mentioned situations, Rauvolfia serpentina has been considered in this study to evaluate the ability of it in Mother tincture ( $\theta$ ) and different liquid potencies (6C, 12C, 30C, 200C and 1M) to inhibit E. coli in vitro with reference to Complete Repertory 4.5 by Roger van Zandvoort, under the Chapter: STOOL ► CULTURE ► Coli. [12] In this experiment Homoeopathic medicine have shown better growth inhibition zone (GIZ)

than Vehicle control (ethanol 90%). Although alcohol with strength 60% to 90% v/v has known for its antibiotic properties. [13] This is the evidence that zone of inhibition of the bacteria, E. coli by Homoeopathic medicine was not due to the ethanol, some distinct dynamic pharmacological actions were behind it.

## CONCLUSION

This antibacterial study results support the concept of the 'Evidence-Based Medicine', it represents that Homoeopathic medicine has specific inhibitory action against E. coli, the homoeopathic medicine Rauvolfia serpentina 6C ( $0.9 \pm 0.1$ ) c.m. showed the best antimicrobial with growth inhibitory action with statistically significant value ("p" value < 0.05) and effective against E. coli to treat various diseases such as Urinary Tract Infection (uropathogenic E. coli), Gastro-enteritis, Neonatal meningitis and also in rare instances Orthopaedic device-associated infection or septic arthritis, Haematogenous myositis, Septicaemia, Mastitis, Haemolytic-Uremic Syndrome (HUS) and Pneumonia as a complementary and alternative medicine to Amikacin. Further studies are required to screen the activity against E. coli in-vivo.

## ACKNOWLEDGMENTS

The author wishes to thank Bharati Vidyapeeth (Deemed to be University) - Homoeopathic Medical College and Hospital

and Bharati Vidyapeeth (Deemed to be University) - Rajiv Gandhi Institute of Information Technology and Biotechnology, Pune- 411043 for providing necessary laboratory facilities and kind support. Author also extends sincere thanks to Dr. (Mrs.) V.A. Pandit, Professor and Head, Department of Pharmacology, Bharati Vidyapeeth (Deemed to be University) - Medical College, Prof. Dr. (Mrs.) Anita S. Patil, P.G. Co-ordinator, Dr. (Mrs.) Manisha P. Gajendragadkar, Professor and Head, Department of Homoeopathic Pharmacy, Bharati Vidyapeeth (Deemed to be University) - Homoeopathic Medical College, Pune- 411043.

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How to cite this article: Mukherjee A, Aswani MA, Jadhav AB et.al. An in-vitro study to evaluate the anti-bacterial activity of rauwolfia serpentina against escherichia coli. Int J Health Sci Res. 2019; 9(12):39-43.

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