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In vitro evaluation of the antibacterial activity of *Thuja occidentalis* and its potencies (30 and 200) against *Escherichia coli*

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Abstract
Gram-negative *Escherichia coli* (*E. coli*) bacteria are generally considered safe and a natural component of the gut flora. However, certain strains can occasionally cause intestinal and extra intestinal diseases in humans. For nearly two decades, homoeopaths have worked to establish the remarkable potential of minute doses. Conversely, antimicrobial resistance to various infections presents a significant challenge to biomedicine, with some strains exhibiting resistance to multiple medications. Studies indicate that bacterial isolates are typically resistant to most antibiotics (51.1-91.2%), except for nitrofurantoin, which has a lower resistance rate of 7.3%.
The aim of the current study is to evaluate the effects of *Thuja occidentalis* in different potencies on *E. coli*. The broader implication of this research is to demonstrate the action of *Thuja occidentalis* on *E. coli* with evidence of the changes occurring *in vitro*. The antibacterial assay is conducted using the Kirby-Bauer method, with Mueller-Hinton (MH) agar as the medium, since it is regarded as the most suitable for routine bacterial susceptibility testing. Homoeopathic medicines, including *Thuja occidentalis* in 30CH and 200CH potencies, were tested against positive and negative controls. When directly assessed using the Kirby-Bauer method, antibiotics and *Thuja occidentalis* 30CH and 200CH solutions exhibited mild to moderate antibacterial activity, producing inhibition zones of 5mm and 8mm, respectively. These findings suggest that homoeopathic medicines of varying potencies may have an effect against this highly virulent bacterium.

Keywords: *In vitro, E. coli, Thuja occidentalis*, Kirby-Bauer method, Mueller-Hinton agar

Introduction
Escherichia coli (*E. coli*) is a Gram-negative bacillus that constitutes a normal component of the intestinal micro biota but can also be implicated in both intestinal and extraintestinal infections in humans [1]. Numerous *E. coli* strains have been identified, with varying pathogenic potential ranging from mild, self-limiting gastroenteritis to severe complications such as renal failure and septic shock. The organism's virulence is largely attributed to its capacity to evade host immune responses and acquire resistance to commonly used antimicrobial agents.
Intestinal infections caused by *E. coli* are categorised based on the specific pathogenic subtypes, including Enterotoxigenic *Escherichia coli* (ETEC), Enterohaemorrhagic *Escherichia coli* (EHEC), also known as Shiga toxin-producing *Escherichia coli* (STEC) and referred to as EHEC/STEC, Enteroinvasive *Escherichia coli* (EIEC), Enteropathogenic *Escherichia coli* (EPEC), and Enteroaggregative *Escherichia coli* (EAEC) [2, 3].
Although the majority of *E. coli* strains are non-pathogenic, certain variants, such as Shiga toxin-producing *E. coli* (STEC), are associated with serious foodborne illnesses. Transmission typically occurs through the ingestion of contaminated food products, including raw or inadequately cooked minced meat, unpasteurised dairy products, and contaminated fresh produce [4, 5]. Pathogenic *E. coli* strains may contribute to a range of clinical conditions, including diarrhoeal disease, urinary tract infections, respiratory infections, pneumonia, and other systemic complications [2, 6].
Emerging evidence suggests that homoeopathic preparations may exhibit antibacterial activity against *E. coli*. Among the homoeopathic interventions evaluated for antimicrobial effects, *Thuja occidentalis* has been identified as a potential therapeutic agent.

The present study aims to assess the antibacterial properties of *Thuja occidentalis* at varying potencies against *Escherichia coli*, utilising standardised *in vitro* methodologies to determine its efficacy.

Materials and Methods

Sample Collection

- The homoeopathic medicines used in this study were obtained from reputable homoeopathic pharmaceutical suppliers.
- *Escherichia coli* strains were procured from the Microbial Type Culture Collection (MTCC).
- Mueller-Hinton (MH) agar, recognised as a high-quality culture medium, was sourced from HI-Media.
- Sensitivity discs, Whatman Filter Paper No. 1 (6 mm in diameter).

Study Setting

This experimental *in vitro* study was conducted at the Microbiology Research Laboratory of Sarada Krishna Homoeopathic Medical College, Kulasekharam.

Methodology

The antibacterial activity of *Thuja occidentalis* was assessed using the Kirby-Bauer disk diffusion method, a standardised procedure for evaluating microbial susceptibility.

Study Groups

The study comprised four experimental groups:

- **AL 01 (Positive Control):** Ciprofloxacin (5 mg dissolved in 5 mL of distilled water) as the standard antibacterial agent.
- **AL 02 (Negative Control):** Ethanol.
- **AL 03:** *Thuja occidentalis* 30CH.
- **AL 04:** *Thuja occidentalis* 200CH.

Preparation and Inoculation of Mueller-Hinton Agar for Antibacterial Assay

Materials

- Nutrient agar medium.
- Mueller-Hinton (MH) medium.
- Sterile filter discs (6 mm in diameter).

Media Preparation

Mueller-Hinton (MH) agar is widely recognised as the optimal medium for routine bacterial susceptibility testing. The medium was prepared by dissolving 38 g of Mueller-Hinton Agar (Hi-Media) in 1000 mL of distilled water. The mixture was autoclaved at 15 LBS pressure and 121 °C for 15 minutes (pH 7.3). The sterilised medium was cooled, mixed thoroughly, and poured into Petri plates at a volume of 25 mL per plate.

Inoculation of MH Plates

A sterile swab was immersed in an *Escherichia coli* inoculum tube and streaked across the MH plate in a back-and-forth motion to ensure uniform bacterial distribution. Four Whatman filter paper discs (6 mm in diameter) were placed onto the agar surface.

The positive control (PC), Ciprofloxacin, was prepared by dissolving 15 mg in 300 µL of sterile water (5 µg/µL). A 10 µL aliquot of Ciprofloxacin was applied to the designated disc, while 10 µL of Ethanol served as the Negative Control (NC).

Additionally, 10 µL of homoeopathic medicine *Thuja occidentalis* 30CH and 200CH were deposited onto the remaining discs.

Incubation and Zone Measurement

The inoculated plates were incubated at 37 °C for 24 hours. Following incubation, the zones of inhibition including the diameter of the discs were examined and measured in millimetres using a transparent ruler. The absence of an inhibition zone was interpreted as a lack of antibacterial activity.

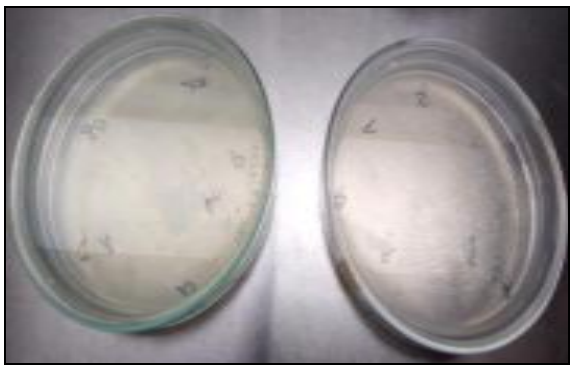

Fig 1: Muller Hinton Agar (MHA) plates

Muller Hinton Agar (MHA) Plate Layout

- **PC:** Ciprofloxacin
- **NC:** Ethanol

Test Groups

- *Thuja occidentalis* 30CH
- *Thuja occidentalis* 200CH

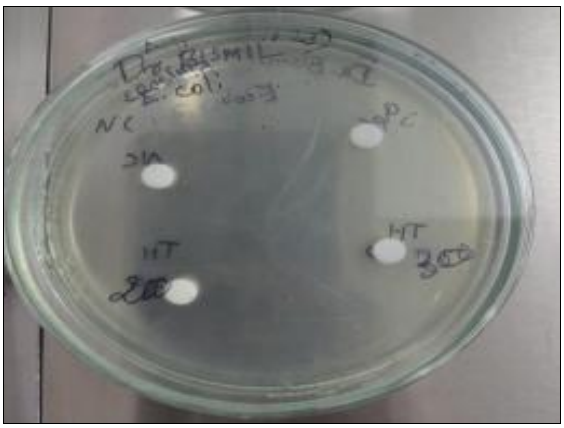

Fig 2: Muller Hinton Agar (MHA) plates after suspending medications

Results

The inhibitory activity of *Thuja occidentalis* 30CH and 200CH against *Escherichia coli* was assessed following 24 and 48 hours of incubation. Among the tested potencies, *Thuja occidentalis* 200CH exhibited a larger zone of inhibition (8 mm), which was comparable to the inhibition zone produced by the standard antibacterial agent, Ciprofloxacin. In contrast, *Thuja occidentalis* 30CH demonstrated a smaller inhibition zone of 5 mm, while ethanol, used as the negative control, exhibited an inhibition zone of 3 mm. The results suggest that *Thuja occidentalis* 200CH demonstrated superior efficacy in inhibiting the

growth of *E. coli* compared to the 30CH potency. These findings provide evidence supporting the potential antibacterial activity of *Thuja occidentalis* 200CH, showing a promising inhibitory effect that approaches the efficacy of conventional antibacterial agents.

Table 1: Antibacterial activity of *Thuja occidentalis* Against *Escherichia coli* after 24 and 48 hours of incubation

Sl. No	Experimental Group	Zone of Inhibition (mm) at 24 Hours
1	Ciprofloxacin (Positive Control)	13
2	Ethanol (Negative Control)	3
3	*Thuja occidentalis* 30CH	5
4	*Thuja occidentalis* 200CH	8

This table summarises the antibacterial efficacy of *Thuja occidentalis* in different potencies against *E. coli*, compared with Ciprofloxacin as a positive control and ethanol as a negative control. The inhibition zones were measured after 24 hours of incubation, demonstrating that *Thuja occidentalis* 200CH exhibited a larger inhibition zone (8 mm), indicating greater antibacterial activity than the 30CH potency (5 mm).

Fig 3: Muller Hinton Agar (MHA) plates 24 hours after incubation

DISCUSSION

Previous studies have utilised similar methodologies to assess the antibacterial effects of various homoeopathic medicines at different potencies. One study investigating *Pyrogenum*, a homoeopathic remedy, reported negligible inhibition at the 200C potency, whereas the 30C potency demonstrated mild to moderate inhibition of microbial activity. Another study evaluating *Rauwolfia* at 6C, 30C, and 200C potencies yielded inhibitory results comparable to those observed in the present study.

Homoeopathic medical science is founded on a vitalistic-substantialistic perspective, considering both universal principles and medical phenomena. According to Hahnemann's Aphorism 81, "this extremely ancient infecting agent, PSORA", refers to a fundamental pathological process. The secondary manifestations of PSORA, previously described in historical pathological texts under various disease classifications, contribute to the progression of chronic conditions. This interpretation underscores the concept that pathological symptoms are not only the external expression of disease but also the result of underlying miasmatic disturbances.

From this standpoint, different pathological classifications associated with bacterial and fungal infections primarily stem from a disruption in the vital force, particularly influenced by miasmatic forces such as psora. The findings of this study indicate that *Thuja occidentalis* 200C and 30C demonstrated antibacterial activity against *Escherichia coli* under standard *in vitro* conditions, supporting homoeopathy's single-remedy approach to treating the whole body. This study highlights variations in bacterial susceptibility corresponding to different potencies of *Thuja occidentalis*.

One of the most frequently debated topics in homoeopathy is whether its therapeutic effects are genuine or merely a placebo response. The results of this study contribute to existing evidence supporting the efficacy of homoeopathic medicines in antibacterial applications. Furthermore, these findings provide valuable insights for the standardisation of homoeopathic drug formulations in the treatment of infections.

Stuart Close postulated that the foundation of homoeopathic prescriptions is based on the totality of symptoms, which encapsulate the therapeutic concept. This principle is applicable in pathology, wherein the full spectrum of symptoms observed in a disease reflects the pathological whole. Conceptually, this entirety can be personified or visualised as an individual pathological entity.

However, the present study did not explore the precise mechanisms by which homoeopathic medicines inhibit the growth of human pathogenic *E. coli in vitro*, warranting further investigation. Additionally, the study did not identify the active compounds present in the homoeopathic formulations, a limitation that should be addressed in future research.

Summary

The antibacterial activity of *Thuja occidentalis* at potencies of 30CH and 200CH was evaluated using the Kirby-Bauer disk diffusion method against *Escherichia coli*. The findings indicated that *Thuja occidentalis* exhibited moderate inhibitory effects, with the 200CH potency producing a zone of inhibition of 8 mm and the 30CH potency yielding a zone of 5 mm. Comparatively, these results suggest that homoeopathic *Thuja occidentalis* demonstrates antibacterial activity similar to conventional antibiotics *in vitro*.

The study was conducted using Mueller-Hinton (MH) agar, recognised as the preferred medium for routine bacterial susceptibility testing. The MH agar was prepared by dissolving 38 g of Mueller-Hinton Agar Medium (Hi-Media) in 1000 mL of distilled water, followed by autoclaving at 15 lbs pressure at 121°C for 15 minutes (pH 7.3). After cooling and thorough mixing, the medium was dispensed into Petri plates (25 mL per plate).

To inoculate the plates, a sterile swab dipped in *E. coli* inoculum was used to streak the MH agar uniformly. Four Whatman filter paper discs (6 mm in diameter) were placed onto the plates, serving as test sites for antimicrobial activity assessment. Ciprofloxacin, used as the positive control (PC), was prepared by dissolving 15 mg in 300 µL of sterile water (5 µg/µL). A 10 µL aliquot of Ciprofloxacin and 10 µL of ethanol, serving as the negative control (NC), were applied to designated discs. Additionally, 10 µL of *Thuja occidentalis* 30CH and 200CH were applied to the remaining discs. Following incubation at 37 °C for 24 hours, the inhibition zones including the diameter of the

discs were measured using a transparent ruler. Absence of an inhibition zone was interpreted as a lack of antibacterial activity.

Conclusion

The findings demonstrated that *Thuja occidentalis* 200CH exhibited moderate antibacterial activity, producing an inhibition zone of 8 mm, while the 30CH potency showed mild activity with a 5 mm inhibition zone. Both potencies displayed greater antibacterial effects than the negative control (3 mm), suggesting a potential inhibitory effect against bacterial growth *in vitro*.

However, the study did not investigate the specific mechanisms through which homoeopathic medicines exert antibacterial effects against *E. coli*. Further research is required to elucidate the underlying principles of homoeopathic antimicrobial activity and to identify the active compounds present in the formulations.

Conflict of Interest: There is no conflict of interest

Financial Support: Not available

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