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Exploring The Antimicrobial Potential Of Homoeopathic Medicine Terebinthinae Oleum Against UTI Causing Escherichia Coli

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Abstract: Urinary tract infections (UTIs) are a prevalent health concern particularly among women, and are primarily caused by the bacterium Escherichia coli (E. coli). The increasing resistance of E. coli to conventional antibiotics poses a significant challenge to the effective treatment of UTIs. Homoeopathic medicine, with its holistic approach and minimal side effects, has garnered interest as a potential alternative or complementary therapy in treating infections. Terebinthinae Oleum, commonly known as Oil of Turpentine, has been traditionally used for its purported antimicrobial properties. The Homoeopathic preparation of this drug is known to have a beneficial action in urinary complaints like UTIs. The study aims to investigate the antimicrobial potential of the Homoeopathic Medicine Terebinthinae Oleum against E. coli strains responsible for UTIs and to evaluate the most effective homoeopathic potency against the growth inhibition of E.coli strains. Two methods were used: The first method was Kirby Bauer Disk Diffusion Method where the pure culture of E. coli was inoculated in the Muller Hinton Agar (MHA) plate and the discs were impregnated with the Terebinthinae oleum 30, 200, 1M, and the control (Ethanol 90%) for 24 hours at 37°C. After 24 hours the zone of inhibition around the discs were measured. The second method was Broth Dilution Assay, in this, 0.2 ml of cultured E.coli was added to 5ml of nutrient broth placed in 20 test tubes. Different concentrations (0.2, 0.4, 0.6, 0.8, and 1 ml) of Terebinthinae oleum, 30CH, 200CH, and 1M potencies were introduced into five test tubes each and incubated for 24 hours at 37ºC. The microbial growth was assessed by measuring turbidity. The Minimum Inhibitory Concentration (MIC) was measured by recording the optical density (OD) at 630 nm in each test tube using a UV-Vis spectrophotometer. In disk diffusion method, the zone of inhibition around discs of Terebinth 30, 200, 1M and ethonal was 8mm, 6.4mm, 7mm, and 6mm respectively. While in the Broth Dilution Assay, the OD at 630nm for 1ml of Terebinthinae oleum 30 was 0.0353 which indicates the MIC. The conclusion is that homoeopathic medicine Terebinthinae oleum can effectively control the growth of E.coli in 30CH potency.

Keywords: E. coli, Homoeopathy, Antimicrobial, UTI, Terebinthinae oleum, In Vitro

Introduction:

Urinary tract infections (UTIs) are one of the most common health concern, particularly among the women, often caused by the gram negative flagellate bacterium Escherichia coli (E. coli). [1][2][3] Urinary tract infections fall under the category of extraintestinal infections and the leading cause is the presence of uropathogenic E. coli (UPEC). [4][5] UPEC can lead to serious complications like acute renal failure and uses virulence factors to breach the urinary tract's defenses, triggering immune responses involving cytokine

production and neutrophil recruitment. Some UPEC strains can suppress these immune responses, allowing persistent infections and recurrences. Understanding these mechanisms is key to preventing UTIs and related complications.[6] The increasing resistance of E. coli to conventional antibiotics poses a significant challenge to the effective treatment of UTIs, highlighting the urgent need for alternative therapeutic options. In the clinical practice, Cantharis is one of the most frequently used homoeopathic medicine for UTIs and found to have potent antimicrobial activity against E.coli.[7] Apart from this many other remedies like Cantharis, Equesitum, Sarsaparilla, Apis mel, Lyco, Staphysagria, works beneficial for UTIs.[8] One such remedy, Terebinthinae Oleum, commonly used in homeopathy for kidney and bladder-related ailments. Terebinthinae Oleum belongs to the Pinaceae family is also known as "turpentine oil" obtained by distillation of oleo-resin. It has long been used in Iranian traditional medicine as an aseptic agent in the creation of ointments.[9] In homoeopathy, Terebinthinae Oleum is indicated for urinary conditions such as strangury with bloody urine, suppressed or scanty urine with a violet odor, and painful urethritis. It is useful in cases of inflamed kidneys, particularly after acute diseases, and nephritis following skin irritation. Symptoms include frequent urges to urinate, burning and drawing pain in the kidney region, spasms during attempts to urinate, and haematuria with painful dysuria. The urine may be scanty, bloody, or have a thick, slimy, yellowish sediment, often with a strong violet odor.[10] This article explores an in vitro study investigating the antimicrobial potential of Terebinthinae Oleum against UTI-causing strains of E. coli, with a focus on evaluating the most effective homeopathic potency.

Materials & Methods:

Materials:

Microorganism: Pure culture of E. coli was procured from MTCC.

Media: The culture medium MHA was purchased from Hi-Media.

Homoeopathic medicine: Terebinthinae Oleum 30, 200, and 1M was purchased from Willmar Schwabe pharmaceutics.

Methods:

Study setting: The study was conducted in the Sarada Krishna Homoeopathic Medical College Research Laboratory, Kulasekharam.

Study design: Experimental study design

Methodology:

1. **Kirby Bauer method:** Escherichia coli was grown as a broth culture in nutritional broth and was pure cultured on Muller Hinton Agar Medium. After that, Muller Hinton agar media was used to create a bacterial lawn in a petri dish. After allowing the bacterial colonies to develop, the environment's modifications and the bacteria's growth are evaluated. When the bacterial colonies have fully formed, they are exposed to homoeopathic medicine's effects. The control group, which received medication in a plain disc, and Terebinthinae Oleum. The medicated disc and the petri dish with the bacterial colonies was incubated at 370C for 24 hours with three different potencies—30CH, 200CH, and 1M—along with an alcoholic control. By evaluating the zone of inhibition, the antibacterial efficacy of the potencies and the control on bacteria was evaluated. We measured, tallied, and compared the zone of inhibition. The effectiveness of the medications was therefore determined once the research was completed in triplicates.

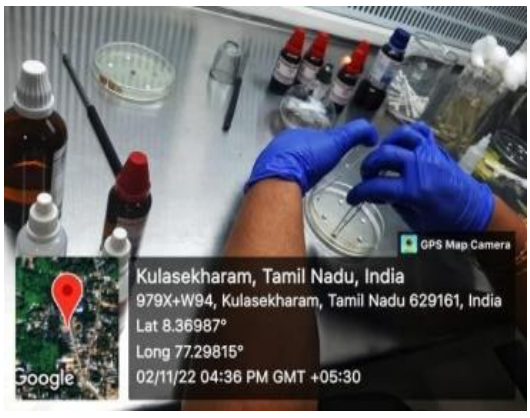

Fig. 1. Disk diffusion method using Terebinthinae oleum in different potencies

2. **Mic- broth dilution assay:** In this method, 5 ml of nutrient broth (1.5 g in 100 ml distilled water) was placed in 20 sterilized test tubes. After cooling, 0.2 ml of cultured E.coli was added to each test tube. Different

concentrations (0.2, 0.4, 0.6, 0.8, and 1 ml) of Terebinthinae oleum, 30CH, 200CH, 1M potencies and control were introduced into five test tubes each. The tubes were incubated for 24 hours at 37ºC, and microbial growth was assessed by measuring turbidity. The optical density was recorded at 630nm using a UV-Vis spectrophotometer.

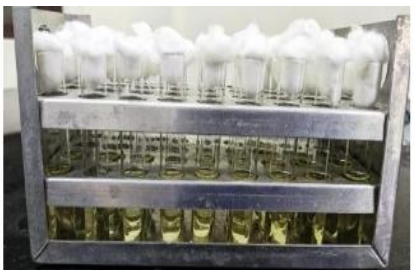

Fig. 2. Preparation of nutrient broth

Fig. 3. Incubated nutrient broth

Observation & Results:

The zone of inhibition was measured to obtain the results of disk diffusion method. (Fig.4). The results of the Kirby Bauer Disk Diffusion method revealed varying zones of inhibition for the different potencies of Terebinthinae Oleum. (Table 1.).

Fig. 4. Zone of inhibition measurement

Table 1. Antimicrobial activity of Terebinthinae oleum 30CH, 200CH & 1M against Escherichia coli.

Sl. No.	Sample	Zone of inhibition in mm
1	Terebinthinae 30CH	8mm
2	Terebinthinae 200CH	6.4 mm
3	Terebinthinae 1M	7mm
4	Ethanol (control)	6mm

The Relative Growth Inhibition Percentage (RGIP) is a measure used to evaluate the effectiveness of a substance (e.g., a drug or compound) in inhibiting the growth of bacteria or other organisms relative to a control.

Table 2. Relative growth inhibition percentage of different potencies of Terebinthinae oleum

Sl. No.	Sample	Relative growth inhibition percentage (%)
1	Terebinthinae 30CH	33.3%
2	Terebinthinae 200CH	0%
3	Terebinthinae 1M	16.6%

In the Broth Dilution Assay, the Minimum Inhibitory Concentration (MIC) was identified by measuring the OD of each test tube. The significance of MIC is indicated by values below 0.1; which was noticed in the 30CH potency of Terebinthinae oleum, where the OD for the 1ml concentration was 0.0353 at 630nm, indicating significant inhibition of E. coli growth. This value was considered the MIC, as it reflected the

lowest concentration of Terebinthinae Oleum required to inhibit visible bacterial growth. The 200CH and 1M potencies exhibited less pronounced antimicrobial effects, with higher OD readings, suggesting that Terebinthinae 30CH was the most potent and effective against E. coli. (Table 3.)

Table 3. MIC in different concentrations.

Terebinth	0.2ml	0.4ml	0.6ml	0.8ml	1.0ml
30CH	0.4603	0.3711	0.2488	0.151	0.0353
200CH	0.4338	0.3525	0.2901	0.1908	0.1379
1 M	0.4375	0.3248	0.2791	0.154	0.0628

This study emphasizes the existence of antibacterial activity in Terebinthinae Oleum 30CH in comparison to the control as well as modest antibacterial alterations in 1M potency and 200th potency. Thus, it disproved the theories that Terebinthinae Oleum possesses antibacterial properties that will inhibit the growth of germs.

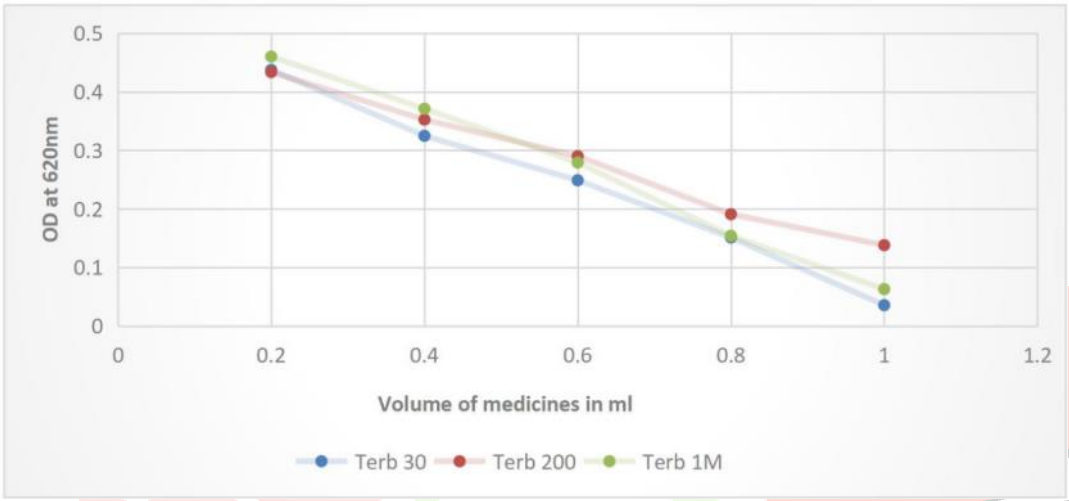

Fig. 5. Graphical representation of MIC in different ml of medicine.

Discussion:

The study highlights the antimicrobial potential of Terebinthinae Oleum against UPEC-induced UTIs. Among the tested potencies, Terebinthinae 30CH exhibited the most significant antimicrobial activity, as demonstrated by both the disk diffusion and broth dilution assays. The Kirby Bauer Disk Diffusion Method showed that Terebinthinae 30CH produced the largest zone of inhibition (8mm), indicating that this potency is most effective in preventing bacterial growth on solid media. Similarly, the Broth Dilution Assay confirmed that the MIC for Terebinthinae 30CH was at 1ml, with an OD of 0.0353, suggesting this concentration was sufficient to inhibit E. coli in a liquid environment. In a previous in vitro study against E.coli, the results of disk diffusion method showed that pyrogenium 30CH has a potent growth inhibitory action.[11] Another study revealed that Cantharis 30CH and mother tincture had antimicrobial action but the zone of inhibition was more in 30CH potency.[7] These studies supports the effective antimicrobial action of Homoeopathic medicines in 30CH potency. This finding suggests that homeopathic remedies like Terebinthinae Oleum may offer a viable complementary approach in the management of UTIs, particularly for patients seeking alternatives to conventional antibiotics.

Conclusion:

This in vitro study provides valuable insights into the antimicrobial potential of Terebinthinae Oleum against E. coli strains associated with UTIs. The results indicate that Terebinthinae 30CH exhibits the most potent antimicrobial activity, offering a promising alternative for managing bacterial infections, particularly in light of growing concerns over antibiotic resistance. Further clinical studies are necessary to validate these findings and explore the practical application of Terebinthinae Oleum in treating UTIs in patients.

Nonetheless, this study marks an important step towards understanding the role of homeopathy in combating microbial infections.

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