International Journal of Homoeopathic Sciences

E-ISSN: 2616-4493
P-ISSN: 2616-4485
www.homoeopathicjournal.com
IJHS 2024; 8(1): 328-330
Received: 01-12-2023
Accepted: 05-01-2024

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An *in-vitro* study to evaluate the anti-bacterial activity of cantharis in various potencies against uropathogenic *Escherichia coli*

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DOI: https://doi.org/10.33545/26164485.2024.v8.i1e.1089

Abstract
Background: In the human intestine, *Escherichia coli*, also known as *E. Coli*, is a prevalent bacterium. The specific strain of *E. Coli* responsible for Urinary Tract Infections (UTIs) is referred to as Uropathogenic *E. Coli* (UPEC). Recent results from the Global Antimicrobial Surveillance System (GLASS) conducted by the WHO reveal a troubling prevalence of antibiotic resistance, with *E. Coli* standing out as the most frequently documented resistant bacteria. Cantharis is one of the most profoundly prescribed Homoeopathic medicines in case of UTIs in clinical practice. This study focuses on assessing the *in vitro* antibacterial action of the Homeopathic medicine Cantharis against Uropathogenic *E. Coli*.
Materials and Methods: The Homoeopathic Medicine Cantharis in Q, 30 C, 200 C, and 1M was tested for antibacterial activity using the Kirby-Bauer Disk Diffusion test. The colonies of the uropathogenic strain of *E. Coli* were cultured in nutrient broth and inoculated on Muller-Hinton Agar plates. The discs impregnated with Homoeopathic medicine Cantharis in Q, 30 C, 200 C, 1M and Vehicle control (Ethanol 90%) were placed in MHA plates and left for 24 hours at 37 ºC after which the zone of inhibition was measured.
Results: The zone of growth inhibition around Cantharis Q, 30C, 200 C, 1 M, and control was (1.6±0.1) c.m., (1.6±0.1) c.m., (0.8±0.1) c.m., (0.6±0.1) c.m. and 0.6 c.m. respectively.
Conclusion: Cantharis Q and 30C exhibited the maximum Growth Inhibitory Zone against *Escherichia coli*, compared to the control.

Keywords: Anti-bacterial, bacteriolytic, cantharis, *Escherichia coli*, homoeopathy

Introduction
A gram-negative, facultatively anaerobic bacterium that is frequently found in the colon is called *Escherichia coli* [1]. Though they are generally harmless, they can cause various health issues among which UTI is the commonest disease which is caused by the Uropathogenic strains of *E. Coli*. Other diseases such as Gastroenteritis, Neonatal meningitis, and in rare cases, Septicaemia, Mastitis, Pneumonia etc are caused by different strains of *E. Coli* through the feco-oral route [2, 6]. A urinary tract infection (UTI) is characterized by the detection of a specific bacterial count in the urine, typically exceeding 105/ml. In clinical terms, UTIs are classified as either uncomplicated or complicated based on the existence of structural or neurological abnormalities within the urinary tract. Symptomatic UTIs are categorized by severity, including urosepsis syndrome, pyelonephritis (an upper UTI involving kidney infection) which occurs when the bacteria ascends to the kidneys through ureters from the biofilm in the urinary bladder, and cystitis (a lower UTI involving bladder infection) when the colonization of bacterium is limited to the bladder alone [3, 4]. Approximately 150 million individuals globally experience urinary tract infections (UTIs) annually, resulting in significant societal burdens. An estimated 40% of women encounter at least one UTI throughout their lives, and around 11% of women aged 18 and above suffer from a UTI episode each year [5].

Antibiotic resistance of *E. Coli*
After the invention of the antibiotic Penicillin by Alexander Fleming the infectious diseases were under control. However, due to the extensive use of antibiotics, the pathogenic microbes developed drug resistance against them [9].

The WHO has introduced a Global Priority Pathogens List (Global PPL) and the GLASS to prioritize research and development of new antibiotics. The global PPL categorizes antibiotic-resistant bacteria into three priority tiers (I, II, III): Critical, High and Medium. Enterobacteriaceae species, which includes *E. Coli*, are in Priority I (Critical). GLASS indicates widespread antibiotic resistance, with *E. Coli* being the most commonly reported resistant bacteria. Across countries, the proportion of patients with bacteria resistant to commonly used antibiotics varies significantly, ranging from 8% to 65% [10, 11]. In a retrospective study conducted at a Mumbai tertiary healthcare hospital to assess current antibiotic sensitivity patterns in uropathogens causing UTIs, the data revealed an overall UTI prevalence of 33.54%, with 66.78% in females and 33.22% in males. Prevalence was higher in females, particularly those aged 31 to 45, and in males above 45. *Escherichia coli* (53.77%) and *Klebsiella pneumoniae* (27.40%) were the main isolates. Effective antibiotics included meropenem, gentamicin, nitrofurantoin, and cotrimoxazole, while resistance was higher in fluoroquinolones, amoxicillin, and third-generation cephalosporins. The findings highlight the evolving drug resistance landscape, emphasizing the necessity for regular surveillance to update physicians for more precise empirical UTI treatment strategies [8].

Homoeopathy for UTI

Homoeopathy is the second most commonly used complementary medicine across the world. The Homoeopathic Medicine Cantharis versicatoria is widely used in the treatment of UTI although many other remedies have been used. In a Randomized Double-Blind Placebo Controlled Trial of Cantharis Vesicatoria in Urinary Tract Infection, 30 patients in the treatment group received Cantharis vesicatoria, while those in the placebo group were administered a placebo mother tincture for 6 months. The findings revealed a significant outcome for Cantharis vesicatoria, with a p-value of 0.05. Consequently, Cantharis vesicatoria may be regarded as a viable supplement for treating urinary tract infections [12]. Another case study highlights the successful use of Cantharis 30CH, in Acute Urinary Tract Infection (UTI) in a 4-month-old infant. The parents, hesitant about antibiotics, opted for homoeopathic treatment for their infant. The Homoeopathic medicine Cantharis, dissolved in breast milk, was administered thrice daily and produced noticeable improvement within three days and complete resolution of symptoms in six days. The results suggest that a well-chosen Homoeopathic simillimum, in the right potency and dosage, can effectively address *E. Coli* infections even in infants [13].

Indications of Cantharis for Urinary complaints

Urinary retention with cramp-like bladder pains. Urgent but ineffective attempts to urinate, painful emission drop by drop. Difficulty in urination, weak and scattered stream. Increased urine secretion. Pale yellow or deep red-colored urine. Flow of sanguineous mucus and blood drops from the bladder. Purulent urine. Burning smarting sensation during urination. Incisive pains in the front part of the urethra during and after urination. Sharp, tearing, incisive pains, successive pullings, and pulsations in urinary organs. Burning, stinging, and tearing sensations in the kidneys. Pressing pain in kidneys extending to the bladder, ureters; relieved by pressing on the glans. Inflammation and ulceration of kidneys, bladder, and urethra. Exceedingly painful sensitivity in the bladder region when touched [14].

Materials and Methods

The antibacterial efficacy of the sample was assessed using the disk diffusion method on the Muller Hinton agar (MHA) medium. A variety of non-fastidious organisms can be grown in Mueller-Hinton agar, which is a non-selective and non-differential media. A mixture of 3.8 gm of Muller Hinton agar was dissolved in 100ml of distilled water, supplemented with 1gm of agar, and subjected to sterilization. Subsequently, the sterilized medium was poured into sterile petri plates and allowed to solidify for one hour. After solidification, sterile swabs moistened in *E. Coli* bacterial suspension were used to spread the inoculates on the solid MHA plates. Plain discs were impregnated with varying potencies (30C, 200C, 1M) and the mother tincture of Cantharis, along with the vehicle control (ethanol 90%). These prepared discs were then placed on MHA plates and incubated for 24 hours at 37 ºC. The extent of microbial growth inhibition was determined by measuring the diameter of the zone of inhibition surrounding the discs. The zone of inhibition around the wells was measured to screen the antibacterial activity of *E. Coli*.

Results

The zone of growth inhibition around Cantharis Q, 30C, 200C, 1M, and control was (1.6±0.1) c.m., (1.6±0.1) c.m., (0.8±0.1) c.m., (0.6±0.1) c.m. and 0.6 c.m. respectively.

Table 1: Antibacterial activity of Homoeopathic medicine Cantharis against *E. Coli*

Homoeopathic medicine	Zone of Inhibition		
	Set I	Set II	Set III
Cantharis Q	1.7 cm	1.6 cm	1.5 cm
Cantharis 30 CH	1.7 cm	1.6 cm	1.6 cm
Cantharis 200 CH	0.8 cm	0.7 cm	0.6 cm
Cantharis 1M	0.6 cm	0.6 cm	0.6 cm
Control (Ethanol)	0.6 cm	0.6 cm	0.6 cm

Fig 1: Antibacterial activity of Cantharis against *E. Coli*

Discussion

The objective of this study was to assess the *in vitro* antibacterial effectiveness of Homeopathic Medicine Cantharis in Mother tincture (Q) and various liquid potencies (30 C, 200 C, and 1M) against uropathogenic *E. Coli* strain. The experimental results revealed that Homeopathic medicine exhibited a more significant reduction in the growth of the bacteria in Q, and 30CH compared to the Vehicle control (90% ethanol). Despite the well-known antibiotic properties of alcohol with strengths ranging from 60% to 90% v/v, the observed antibacterial activity of Homeopathic medicine surpassed that of the ethanol control. This supports the notion that the observed zone of inhibition of *E. Coli* bacteria by Homoeopathic medicine was not attributed to ethanol; rather, some

dynamic pharmacological actions were responsible. In a study conducted using a cystitis-induced mice model to investigate the efficacy of the Homoeopathic medicine Cantharis against UPEC. In this study, Cantharis 6cH or a placebo was administered through drinking water (1:100) for 24 hours post-infection in 24 adult susceptible female BALB/c mice. The results indicated that Cantharis 6 CH induced specific local immune modulation, altering the balance of inflammatory cells and cytokines in the bladder and pelvis mucosa [15].

Conclusion

The results of this *in vitro* antibacterial study indicate that Homoeopathic medicine Cantharis Q and 30 CH exhibits a distinct growth inhibitory effect on uropathogenic *E. Coli* while Cantharis 200 CH showed minimal growth inhibitory effect. This supports the application of Homeopathic Medicine Cantharis for treating Urinary Tract Infections, given its ability to inhibit the growth of the bacteria.

Conflict of Interest

Not available

Financial Support

Not available

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How to Cite This Article

Sibin RA, Manikutty VS, Mugalyaa PR, Muthumari M, Nayana MR, Vidya V. An *in-vitro* study to evaluate the anti-bacterial activity of cantharis in various potencies against uropathogenic *Escherichia coli*. International Journal of Homoeopathic Sciences. 2024;8(1):328-330.