

**IN-VITRO EVALUATION OF ANTI-MICROBIAL POTENTIAL OF TRADITIONAL SIDDHA FORMULATION SEENTHIL CHOORANAM AGAINST UROPATHOGEN BY DISC-DIFFUSION TECHNIQUE**

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**ABSTRACT**

Urinary tract infections (UTIs) are one of the most frequent clinical bacterial infections in women, accounting for nearly 25% of all infections. Around 50–60% of women will experience a UTI in their lifetime. Increasing antimicrobial resistance has stimulated interest in non-antibiotic prophylaxis of recurrent UTIs. Well-known steps in the pathogenesis of UTIs are urogenital colonization and adherence of uropathogens to uroepithelial cell receptors. *Escherichia coli* (*E. coli*) is an opportunistic pathogens that cause severe and life-threatening infections in immunocompromised patients. In women with recurrent UTIs an increased adherence of *E. coli* to urogenital epithelial cells was seen compared to healthy controls. Antibiotics are essential for the

control and treatment of *E. coli* infections in humans and animals. However, it is generally accepted that antimicrobial resistance is associated with the quantity of antibiotic consumption. The worldwide emergence of multidrug-resistant bacteria has dramatically

limited the number of antibiotics that retain activity against these pathogens. This problem has been further amplified by the dearth of novel classes of antibiotics. Therefore, development of novel therapeutic strategies for infectious diseases is high demand. Siddha system of medicine has excellent antimicrobial drugs with combination of bioactive components that's acts synergistically in multiple way thereby chance of resistance is greatly reduced and also several siddha traditional drugs are been prescribed for treating UTI infection one such drug is Seenthil chooranam (SC). The main aim of the present investigation is to evaluate the anti-microbial potential of the SC against *E-coli* a known uropathogen by disc diffusion method. Results of the study has revealed that the drug SC at the concentration of 4000 µg exhibited the maximum zone of inhibition of 10mm when compare to that of the standard with the maximum zone of 12 mm. It was concluded from the data's obtained from the present investigation that the therapeutic value of siddha formulation relies in the various chemical constituent's presents in it. The bioactivity of formulation SC may be attributed to phytochemical a constituent which needs to be studies in details in near future.

**KEYWORDS:** Urinary tract infections, *Escherichia coli*, Antibiotic, Resistance, Antimicrobial, Siddha traditional drugs, Seenthil chooranam.

## 1. INTRODUCTION

Urinary tract infections (UTIs) are a severe public health problem and are caused by a range of pathogens, but most commonly by *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis* and *Staphylococcus saprophyticus*. High recurrence rates and increasing antimicrobial resistance among uropathogen threatens to greatly increase the economic burden of these infections.<sup>[1]</sup> While UTIs are typically considered extracellular infections, it has been demonstrated that uropathogenic *E. coli* can invade and replicate within the bladder cells to form intracellular bacterial communities (IBCs).<sup>[2]</sup> In women with acute uncomplicated symptomatic UTIs, most commonly caused by uropathogenic *E. coli*, IBCs could be detected in exfoliated urothelial cells in about one fifth of urine specimens

Recurrent urinary tract infection (UTI) might be one of the most common problems in urological clinics. Recent research has revealed novel evidence about recurrent UTI and it should be considered a different disease from the first infection. The pathogenesis of recurrent UTI might include two mechanisms, bacterial factors and deficiencies in host defense. Bacterial survival in the urinary bladder after antibiotic treatment and progression to

form intracellular bacterial communities might be the most important bacterial factors. In host defense deficiency, a defect in pathogen recognition and urothelial barrier function impairment play the most important roles. Immunodeficiency and urogenital tract anatomical abnormalities have been considered the essential risk factors for recurrent UTI. In healthy women, voiding dysfunction and behavioral factors also increase the risk of recurrent UTI.<sup>[3]</sup>

*E. coli*, a member of the bacterial family of *Enterobacteriaceae*, is the most prevalent commensal inhabitant of the gastrointestinal tracts of humans and warm-blooded animals, as well as one of the most important pathogens.<sup>[4]</sup> As a commensal it lives in a mutually beneficial association with hosts, and rarely causes disease. It is, however, also one of the most common human and animal pathogens as it is responsible for a broad spectrum of diseases. *E. coli* is present in the Centers for Disease Control and Prevention (CDC) list of biological agents potentially threat to public health and safety.

Rising antibiotic resistance and the scarcity of new antimicrobials has long been acknowledged.<sup>[5,6]</sup> A major challenge in global health care is the need for novel, effective and affordable medicines to treat microbial infections, especially in developing countries of the world, where up to one-half of deaths are due to infectious diseases.<sup>[7,8]</sup> *E. coli* is intrinsically resistant to therapeutic levels of penicillin G, the first  $\beta$ -lactam introduced into clinical practice, because of its outer membrane barrier. *E. coli* is also resistant to several different classes of antibiotics with distinct mechanisms of action.<sup>[9,10]</sup> Worldwide spending on finding new anti-infective agents (including vaccines) is expected to increase 60% from the spending levels in 1993.<sup>[11]</sup> New sources, especially herbal or alternate complimentary sources, are also being investigated.

In the current scenario, there have been increasing antibiotic resistant strains of clinically important pathogens, which have led to the emergence of new bacterial strains that are multi-resistant.<sup>[12]</sup> The non-availability and high cost of new generation antibiotics with limited effective span have resulted in increase in morbidity and mortality.<sup>[13]</sup> Therefore, there is an urgent need to look out for new substances from other sources such as traditional medicines with proven antimicrobial activity. Consequently, this has led to the search for more effective antimicrobial agents of herbal or complimentary origin, with the aim of discovering potentially useful active phytoconstituents that can serve as a source and template for the synthesis of new antimicrobial agents.<sup>[14]</sup> The main aim of the present investigation is to

evaluate the anti-microbial potential of the siddha drug SC against *E-coli* a known uropathogen by disc diffusion method.

## 2. MATERIALS AND METHODS

### 2.1. Source of raw drugs

Ingredients and other raw materials required for the formulations were purchased from a well reputed indigenous drug shop at Chennai. All raw drugs were properly identified and authenticated by the concerning authority before clinical usage.

### 2.2. Ingredients

The formulation *Seenthil chooranam* comprises of the following ingredients

1. Seenthil - *Tinospora cordifolia*
2. Karisalai - *Eclipta prostrate*
3. Earthworm - *Lumbricus terrestris*

### 2.3. Preparation<sup>[15]</sup>

Both the formulation *Seenthil chooranam* were prepared in accordance with the procedure as mentioned in prescribed text of Agasthiyar Vaidhya kaviyam.

### 2.4. Anti-Microbial Evaluation by Disc-diffusion method<sup>[16-18]</sup>

The antibacterial activities of the sample SC were carried out by disc diffusion method. Sample SC was extracted using ethanol by hot- continuous extraction method. The concentrations of the test compounds were used at the concentration of 500, 1000, 2000 and 4000 µg. The target microorganisms were cultured in Mueller–Hinton broth (MHB). After 24 h the suspensions were adjusted to standard sub culture dilution. The Petri dishes containing Muller Hinton Agar (MHA) medium were cultured with diluted bacterial strain. Disc made of Whatman No.1, diameter 6 mm was pre-sterilized and was maintained in aseptic chamber. Each concentration was injected to the sterile disc papers. Then the prepared discs were placed on the culture medium. Standard drug Ciprofloxacin (10µg) was used as a positive reference standard to determine the sensitivity of each microbial species tested. Then the inoculated plates were incubated at 37° C for 24 h. The diameter of the clear zone around the disc was measured and expressed in millimeters as its anti-microbial property.

### 3. RESULT

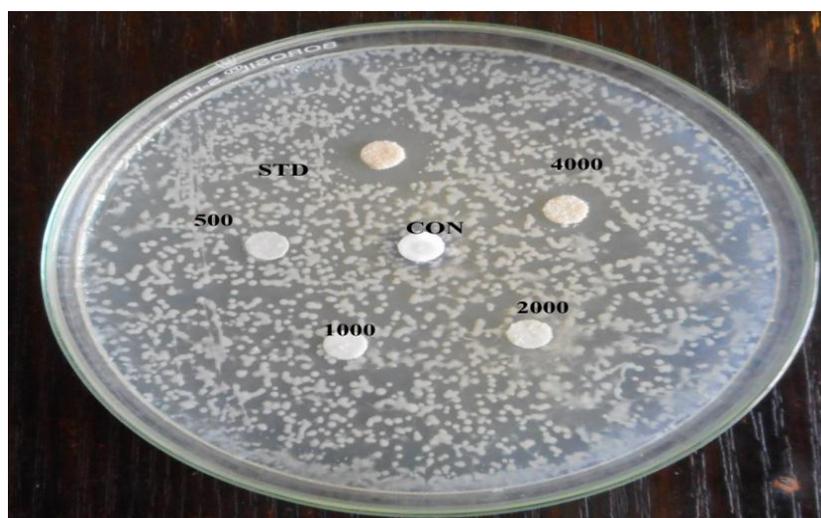
#### 3.1. Effect of formulation SC against uropathogen E-Coli

From the results of the present study it was observed that sample SC is effective tested microorganism *E-coli* with the maximum zone of inhibition of 10mm at 4000  $\mu$ g when compare to that of the standard with the maximum zone of 12 mm. The results were depicted in the table 1 and represented in the figure 1.

**Table 1: Zone of Inhibition data of Anti-Microbial activity of the drug SC.**

Sample	<i>E. Coli</i>			
	500 $\mu$ g	1000 $\mu$ g	2000 $\mu$ g	4000 $\mu$ g
SC	-	-	-	10
Ciprofloxacin (10 $\mu$ g)	12			

- = Not active



**Figure 1: Anti- Microbial Effect of SC against Escherichia coli.**

### 4. DISCUSSION

Urinary tract infections (UTIs) are among the most common types of bacterial infections occurring both in the community and hospital settings.<sup>[19,20]</sup> There are two types of UTIs: hospital associated urinary tract infection (HAUTIs), and community-associated urinary tract infection (CAUTIs).<sup>[21]</sup> Women are the predominant group of patients with CAUTIs. Among bacteria causing UTIS, *E. coli* is considered as the most predominant cause of both community and nosocomial UTIs. Evidence suggest significant relationship between extensive use of antimicrobial and antimicrobial resistance.<sup>[22,23]</sup> Therefore, appropriate antibiotic prescription and usage will reduce the disease burden of UTIs and hence lower its complications and costs.<sup>[24]</sup>

Clinically, UTIs are categorized as uncomplicated or complicated. Uncomplicated UTIs typically affect individuals who are otherwise healthy and have no structural or neurological urinary tract abnormalities<sup>[25,26]</sup>; these infections are differentiated into lower UTIs (cystitis) and upper UTIs (pyelonephritis). Several risk factors are associated with cystitis, including female gender, a prior UTI, sexual activity, vaginal infection, diabetes, obesity and genetic susceptibility. Complicated UTIs are defined as UTIs associated with factors that compromise the urinary tract or host defence, including urinary obstruction, urinary retention caused by neurological disease, immunosuppression, renal failure, renal transplantation, pregnancy and the presence of foreign bodies such as calculi, indwelling catheters or other drainage devices.<sup>[27,28]</sup>

Human pathogenic microorganisms have developed resistance to drugs owing to the extensive use of commercial synthetic antibacterial drugs in large quantity without proper medical prescriptions and tests. This condition has raised alarm in most developed and developing countries and the scientists are forced to search an alternative to these compounds, often in the form of natural medicines from resources such as plants.

Antimicrobial resistance is a major and increasing global healthcare problem.<sup>[29]</sup> Since the introduction of the penicillin, a large number of bacteria have responded to the use of antibiotics with their ability to evolve and transmit antimicrobial resistance to other species.<sup>[30]</sup> Increased consumption of antimicrobial agents and their inappropriate use are among factors which further accelerated this phenomenon. Furthermore, the continuous migration of people between countries as well as international tourism and business travel play an important role in the acquisition and spread of multidrug resistant strains.<sup>[31]</sup>

Clinical microbiologists have enormous reasons to be interested in the topic of antimicrobial from traditional medicine. First, it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians; several are already being tested in humans. It is reported that, on average, two or three antibiotics derived from microorganisms are launched each year.<sup>[32]</sup> After a downturn in that pace in recent decades, the pace is again quickening as scientists realize that the effective life span of any antibiotic is limited. In addition, many people are interested in having more autonomy over their medical care. A multitude of plant compounds (often of unreliable purity) is readily available over-the-counter from herbal suppliers and natural-food stores, and self-medication with these substances is commonplace. In the present study the drug SC at the concentration of 4000 µg

exhibited the maximum zone of inhibition of 10mm when compare to that of the standard with the maximum zone of 12 mm. It was concluded from the data's obtained from the present investigation that the therapeutic value of siddha formulation relies in the various chemical constituent's presents in it. The bioactivity of formulation SC may be attributed to phytochemical a constituent which needs to be studies in details in near future.

## 5. CONCLUSION

Siddha system of medicine has numerous formulations which have naturally occurring compounds found in plants, herbs and spices have been shown to possess antimicrobial activity and serve as a source of antimicrobial agents against several pathogens like *E-coli*. The drug *Seenthil chooranam* is a rich source of natural chemical compounds that have been utilized in various applications in siddha system of traditional medicines. Urinary tract infections are some of the most common bacterial infections. The inappropriate use and misuse of antimicrobials increased the resistance in uropathogens as well as in normal human bacterial flora. Exploration of medicine from alternate traditional source with multiple action and also reduced resistance is of highly essential in treating UTIs. Data's of present investigation has clearly revealed that the drug SC was effective against the tested uropathogen E-coli and this action of the drug may be due to the presence of multiple phytocomponents present with in the formulation which need to be studied individually to explore the exact mechanism of action. Hence it was concluded that the siddha drugs like SC may be considered as a drug of choice for clinical management of UTI.

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