



IN-VITRO ANTIMICROBIAL ACTIVITY OF SIDDHA MEDICINE-KAARA SOODA SATHU PARPAM (KSSP) AGAINST – URINARY TRACT INFECTION (UTI)

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ABSTRACT

Urinary tract infections (UTIs) are a common health issue caused by various pathogens such as *E. coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Proteus mirabilis*, and *Staphylococcus saprophyticus*. These infections occur when bacteria invade the urinary tract, potentially leading to complications such as urinary bladder infections. Risk factors for UTIs include pregnancy, previous infections, age, sexual activity, and poor hygiene. UTIs affect individuals across all socio-economic groups, placing a significant economic burden on families. UTIs are a prevalent healthcare-associated infection, with an estimated 13,000 deaths linked to these infections. While antibiotics are commonly used to treat UTIs, the rise of drug-resistant strains poses a significant challenge to effective treatment. Therefore, there is a growing need for alternative medicine solutions in the face of this global health crisis. Siddha, an ancient system of Indian Traditional Medicine, offers a range of remedies for UTIs. One such formulation, KAARA SOODA SATHU PARPAM(KSSP), was selected for its potential in treating UTIs. In-Vitro Antimicrobial sensitivity testing using the Resazurin Microtitre Assay method demonstrated moderate activity against the pathogen *Staphylococcus aureus* (MIC 1000 µg) and *Pseudomonas aeruginosa* (MIC 500 µg) and significant level of activity against *E-Coli pneumonia* (MIC 125 µg) and *Klebsiella pneumonia* (MIC 250 µg). Similarly, the sample demonstrate consistent anti-fungal activity against *Candida albicans* with the MIC value of 500 µg.

This study provides evidence supporting the use of Siddha medicine KAARA SOODA SATHU PARPAM(KSSP) for UTI infections.

KEYWORDS: Resazurin Microtitre Assay, KAARA SOODA SATHU PARPAM (KSSP), UTI, Drug resistant, Ancient, Potential, Siddha medicine.

INTRODUCTION

Urinary tract infections (UTIs) are infections that can occur in the urethra (urethritis), bladder (cystitis), or kidneys (pyelonephritis) and are one of the world's most common infectious diseases, affecting 150 million people each year. ⁽¹⁾

UTIs are caused by both Gram-negative and Gram-positive bacteria, as well as by certain fungi. The most common causative agent for both uncomplicated and complicated UTIs is uropathogenic *Escherichia coli* (UPEC). For the agents involved in uncomplicated UTIs, UPEC is followed in prevalence by *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B *Streptococcus* (GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida* spp. For complicated UTIs, the order of prevalence for causative agents, following UPEC as most common, is *Enterococcus* spp., *K. pneumoniae*, *Candida* spp., *S. aureus*, *P. mirabilis*, *P. aeruginosa* and GBS. ⁽²⁾ The World Health Organization's global surveillance report on antibiotic resistance indicated that five out of the six WHO regions had more than 50% resistance to third generation cephalosporins in *E. coli* and *K. pneumoniae* in hospital settings. ⁽³⁾ Antibacterial agents are a major part of the treatment for UTI. Due to the side effects associated with antibiotic and drug resistance a need for an alternative therapy is emphasised. A major error in management of UTI has been that most clinicians give too many drugs for too longer period. ⁽¹⁸⁾

PREVALENCE

Urinary tract infections (UTIs) are a common issue among patients receiving treatment outside of hospitals, and the likelihood of developing one increases with age. Statistics show that around 20% of women over the age of 65 experience UTIs, compared to 11% in the rest of the population. Recurrence of the infection within six months is reported by 27% of affected women. Healthcare-associated UTIs (HAUTIs) are the most prevalent type of healthcare-associated infection globally, making up approximately 24% of cases in developing countries. The high rate of UTI recurrence not only causes distress and worry for those affected but also places a financial strain on their families. It is crucial to implement measures that reduce the socio-economic impact of UTIs and prioritize the overall well-being of individuals, both physically and mentally. ⁽⁴⁾ About 1.5 billion infections due to microorganisms have been reported to occur globally each year resulting in approximately 4.6 million deaths. ⁽³⁾

KAARA SOODA SATHU PAMPAM FOR UTI

The ancient healing practices of traditional medicine have long been valued as essential assets in the fight against diseases in today's modern society. A significant portion of the population relies on plant-based remedies from traditional medicine to treat various illnesses, as they are not only effective in providing relief but also come with minimal side effects. Among the various traditional medicine systems, Siddha, an Indian traditional medicine system, has gained global recognition for its more natural and less aggressive approach compared to conventional treatments with harsh side effects.

This study specifically focuses on the effectiveness of selected Siddha formulations in combating Uropathogens, providing insights into the potential benefits of these ancient remedies in modern healthcare. The book "SIKITCHA RATHINA DEEPAM PART-2" by KANNUSAMIPILLAI introduces a Siddha remedy called KAARA SOODA SATHU PAMPAM(KSSP)⁽¹³⁾ for the treatment of UTI, a medical condition involving the urinary system. The process of preparing this remedy seems simple and cost-effective. Siddha remedies not only alleviate the symptoms of the disease but also improve the functioning of the urinary system. As a result, UTI has been selected as the primary focus of IN-VITROANTIMICROBIAL ACTIVITY to offer new and effective treatment options for individuals requiring medical assistance.

AIM & OBJECTIVE

The primary objective of the study is to determine the In-vitro antimicrobial activity of the chosen Siddha formulations KAARA SOODA SATHU PAMPAM (KSSP) using Resazurin Micrititre Assay against the five test uropathogens- E. coli, Staphylococcus aureus, Pseudomonas, Klebsiella and Candida albicans.

MATERIALS AND METHODS

The Siddha formulations KAARA SOODA SATHU PAMPAM was selected from SIKITCHA RATHINA DEEPAM PART-2" by KANNUSAMIPILLAI⁽¹³⁾. The test sample KSSP was prepared after the purification of the ingredients. The formulation was listed in the Table-1

Table:1 Ingredients of KSSP

SL. NO	NAME	BOTANICAL NAME/ CHEMICAL NAME	QUANTITY
1.	Vengaram	Borax,Sodiyam baborate	1 palam (35 gms)
2.	Karpoora silasathu	Gypsum	1 palam(35 gms)
3.	Lemon juice	Citrus limon.linn	Required quantity

Collection of Raw Material

The indigeneous herbal and mineral raw drugs were procured from a reputed raw drug store, identified and authenticated by the Botanist of Government Siddha Medical College, Chennai, (Voucher number GSMC/MB- 630) and HOD of the Department of Gunapadam, Government Siddha

Medical College, Chennai, Tamilnadu – 106, respectively.

PURIFICATION OF RAW DRUGS ^(14,15)

VENGARAM:

The salt is fried in an earthen plate until the moisture content in it completely gets evaporated ⁽¹⁴⁾.

KARPOORA SILASATHU:

Karpoora silasathu was boiled in tender coconut water, then washed and dried ^(15,16).

PREPARATION OF KARA SOODA SATHU PAMPAM ⁽¹³⁾

- ❖ Grind the above 2(Table1) purified raw materials with lime juice.
- ❖ Then make pelletes of grind material and dry it well.
- ❖ Prepare the crucible and its lid with limestone.
- ❖ Then disintegrate the dried pelletes collect into the crucible and sealed with mud cloth and dry it well.
- ❖ After that it incinerated with 6 cow dung cakes.
- ❖ Then collect the inside material of crucible and grind into fine powder with stone mortar and pestle.
- ❖ Then, store it in an air tight glass container.

DOSAGE: 500 mg twice a day for 15 days.

ADJUVANT: Honey.

TEST ORGANISMS:

1. Escherichia coli
2. Staphylococcus aureus
3. Klebsiella pneumonia,
4. Pseudomonas aeruginosa
5. candida albicans are used for anti – microbial tests.

PROCEDURE

Test was carried out in a 96 well Plates under aseptic conditions. A sterile 96 well plate was labelled. Volume of sample in DMSO comprises of 1000 µg was pipetted into the first well of the plate and transferred to subsequent wells by half of its weight until 8th Well. To all other wells 50 µl of nutrient broth was added and serially diluted it. To each well 10 µl of resazurin indicator solution was added. 10 µl of bacterial/ fungal suspension was added to each well. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. The plate was incubated at 37 °C for 24-48 hrs. The colour change was then assessed visually. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value. Standard drug Chloramphenicol (10µg) was used as a positive reference standard to determine the sensitivity of the bacterial species tested. Standard drug Fluconazole (20µg) was used as a positive reference standard to determine the sensitivity of the fungal species tested.

Table:2

Color Pale Pink	(+) Positive Value indicates the pale pink color in the well – Means there is no anti-microbial activity for the sample in that particular well
Color Dark purple	(-) Negative Value indicates the pale Dark purple Color in the well – Means there is good anti-microbial activity for the sample in that particular well

RESULTS

Table:3 Anti-Bacterial Activity- KSSP Growth of inhibition Chart for the Sample and Standard Drug

S. No	Sample/ Microorg anisms	Growth of inhibition										Cultu re
		W-1 1000 µg	W-2 500 µg	W-3 250 µg	W-4 125 µg	W-5 62.5 µg	W-6 31.2 µg	W-7 15.6 µg	W-8 7.8 µg	STD Chloramphenicol (10µg)	DM SO	
1	E-coli	-	-	-	-	+	+	+	+	-	+	+
2	Pseudomonas aeruginosa	-	-	+	+	+	+	+	+	-	+	+
3	Klebsiella pneumonia-KP	-	-	-	+	+	+	+	+	-	+	+
4	Staphylococcus aureus	-	+	+	+	+	+	+	+	-	+	+

Table:4 Anti-Fungal Activity-KSSP

Growth of inhibition Chart for the Sample and Standard Drug

S. No	Sample/ Microorg anisms	Growth of inhibition										Cultu re
		W-1 1000 µg	W-2 500 µg	W-3 250 µg	W-4 125 µg	W-5 62.5 µg	W-6 31.2 µg	W-7 15.6 µg	W-8 7.8 µg	STD Fluconazole (20µg)	DM SO	
1	Candida albicans	-	-	+	+	+	+	+	+	-	+	+

Figure:1 96 Well plate- Anti-Microbial Spectrum Image

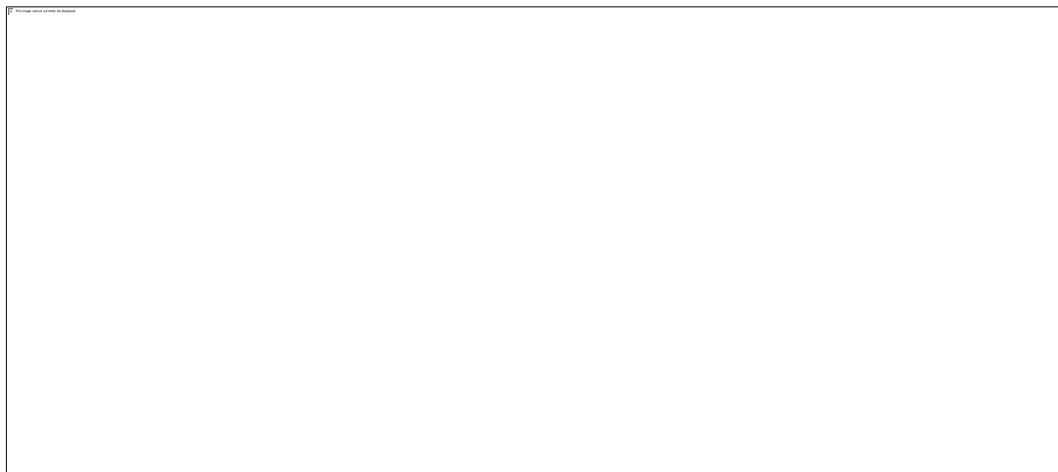


Table:5 Report on MIC (Minimum inhibitory concentration) value of the Sample - KSSP

S. No	Name of the Organism	MIC Value (μg)
1.	E-Coli	125 μg
2.	Pseudomonas aeruginosa	500 μg
3.	Klebsiella pneumonia-KP	250 μg
4.	Staphylococcus aureus	1000 μg
5.	Candida albicans	500 μg

It was observed from the results of the present investigation that the sample reveals convincing In-vitro anti-microbial activity among all tested organisms. It was observed that the sample reveals moderate activity against the pathogen *Staphylococcus aureus* (MIC 1000 μg) and *Pseudomonas aeruginosa* (MIC 500 μg) and significant level of activity against *E-Coli pneumonia* (MIC 125 μg) and *Klebsiella pneumonia* (MIC 250 μg). Similarly, the sample demonstrate consistent anti-fungal activity against *Candida albicans* with the MIC value of 500 μg . (Table5)

DISCUSSION

Urinary tract infection is a common contagion among men and women but the incidence is quite high among women due to their physiology. In simple terms, it can be referred as a condition which women will certainly encounter during the span of their life time. ⁽⁵⁾ UTIs are mainly caused by bacteria, while the involvement of other microorganisms, such as fungi and viruses, is quite rare. the main isolated pathogen is uropathogenic Escherichia coli (UPEC) Candida albicans is the most common type of fungus that causes UTIs. ⁽⁶⁾

Uropathogens possess distinct features such as the ability to produce toxins, siderophores, and adhesins that enable them to adhere to the urinary tract. While urinary tract infections are typically self-limiting, they have the potential to recur and can be transmitted between individuals. Treatment with antibiotics can effectively clear the infection, but it also contributes to the development of antibiotic-resistant strains of uropathogens. Therefore, alternative approaches are crucial in addressing these UTI infections. ⁽⁷⁾

Nearly half of the world's women will experience a symptomatic UTI in their lifetime, and up to one-third of those affected will be plagued by recurrent infections. The management of UTI can be a formidable task given the prevalence of disease and high rate of recurrence, wide range of associated morbidity, rapidly evolving antimicrobial resistance. (8) *Escherichia coli* is responsible for 80–90% of cases of paediatric UTI. The occurrence and severity of this illness are largely mediated by bacterial virulence factors and host defence mechanism. (9) UTIs are also relatively common in children and infants under the age of approximately 2 years and can be acquired both in community settings and in hospitals. Up to the age of 7 years, approximately 5% of girls and 2% of boys experience at least one UTI incident. (12)

Antibiotics will generally produce a “fitness cost” on bacteria, such as slowing their growth rate, but the bacteria can quickly develop mechanisms to compensate. (10) In recent years, there has been a rise in antibiotic-resistant strains of uropathogens. Uropathogenic *E. coli* (UPEC) is one of the bacteria that has become resistant to commonly used antibacterial antibiotics. The worldwide distribution of these resistant strains is largely due to the underuse, overuse, or misuse of antibiotics. Factors contributing to the emergence of these resistant strains include mutation, horizontal gene transfer, and their subsequent spread. (11)

The results of the current investigation indicate that the sample exhibits strong antimicrobial properties against various organisms. The sample showed moderate effectiveness against *Staphylococcus aureus* (MIC 1000 µg) and *Pseudomonas aeruginosa* (MIC 500 µg), and significant activity against *E-Coli pneumonia* (MIC 125 µg) and *Klebsiella pneumonia* (MIC 250 µg). Additionally, it displayed consistent anti-fungal activity against *Candida albicans* with an MIC value of 500 µg (Table 5). Researchers have been focusing on isolating and characterizing antimicrobial compounds from Herbo mineral preparations in recent years. The goal of this study was to demonstrate the efficacy of KAARA SOODA SATHU PARPAM (KSSP) (13) against specific microorganisms causing urinary tract infections. The rapid and global dissemination of antibiotic-resistant bacteria has resulted in the decrease of therapeutic options for many infectious diseases, highlighting the urgent need for new therapies. (17) This medicine could potentially serve as an alternative to antibiotics due to the severe side effects associated with commercially available antibiotics, especially in immunocompromised patients and children. It is strongly recommended to consider natural products for the treatment of infections, as multidrug-resistant pathogens responsible for nosocomial infections pose a significant threat to public health.

CONCLUSION

In summary, the findings of this study clearly demonstrate that Siddha formulations containing KAARA SOODA SATHU PARPAM (KSSP) exhibit good antimicrobial properties against significant Uropathogens. These formulations prove to be a promising option for treating Urinary Tract Infections (UTI) and can be considered as a preferred drug choice for this condition.

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